PARASITOLOGY

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A SUPPLEMENT TO THE

JOURNAL OF HYGIENE

EDITED BY

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Quick Professor of Biology in the University of Cambridge

AND

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THE PROBABLE MODE OF INFECTION AND THE METHODS USED IN CONTROLLING AN OUTBREAK OF EQUINE TRYPANOSOMIASIS (*MURRINA*) IN THE PANAMA CANAL ZONE.

BY S. T. DARLING, M.D.

Chief of Laboratory, Isthmian Canal Commission, Ancon, Canal Zone.

THE methods so successfully used in ridding Panama of yellow fever and delimiting malarial fever have been equally valuable in checking an epidemic of Equine Trypanosomiasis in the Canal Zone.

In 1909 there broke out in the Commission corrals among some mules and a few work-horses a fatal trypanosomal disease which I have identified as *Murrina* or *Derrengadera*, the Panaman names for a fatal disease of horses in this region. The corrals had been free up to this time from any disease of this kind since 1904, the date of the beginning of canal operations by Americans.

The pathogenic agent, *T. hippicum*, has been described elsewhere[1].

My attention was called to the disease some months after its appearance and at a time when from its symptomatology it was regarded by the Veterinarians as "Swamp fever" or the "Infectious Anaemia of Equines," a disease prevalent in some sections of the Western United States, of obscure etiology and whose mode of transmission is unknown.

With the discovery of the trypanosome, infected animals were isolated, and work was begun to ascertain, if possible, the natural means of infection, by an examination of suspected biting flies, ticks, bats and muscid flies. The results of the investigation may be epitomized as follows:

Apparently *ticks* were not responsible, for ticks were never found on mules or work-horses because they were never worked in localities

[1] *Bull. de la Société de Pathologie Exotique*, Paris, III. p. 381, 1910. *American Veterinary Review*, New York, XXXVII. p. 375, 1910.

where they could become infested by ticks. Saddle-horses were the only animals from which ticks could be collected, because they were used out along the trails and other locations where they could, and did, become infested with ticks. For a period of six months, before the true nature of the disease was recognized, the saddle-horses and some of the mules were stabled in a long shed, the stalls of the horses being opposite those of the mules, so that only a few inches of partly open space separated the heads of the horses from the mules. At this time mules kept coming down with the disease, though their near neighbours, the saddle-horses, never became infected. It was concluded from this that ticks did not transmit the disease under these conditions.

Among the biting flies only two species were found within the corral district—*Stomoxys calcitrans* and *Tabanus* sp. *S. calcitrans* was breeding near some of the corrals and could always be taken in the corrals. This fly did not breed in the corral because the horse droppings were removed daily. Very few Tabanids were ever seen within the corrals. A few were taken near the isolation corral and in the new corral at Ancon, which lie on the outskirts of the district near woods, but no Tabanids were ever seen in the old corral at Ancon, which lies within the populated district, nor at the corral at Gatun, which lies away from the woods.

During the height of the epidemic among the mules, several specimens of *S. calcitrans* were taken at the Ancon corral with mule's or horse's blood in their intestinal tracts, and *Stomoxys* could be collected here every day. Now as at this time I had a naturally infected work-horse already under observation and I was confident that horses were as susceptible as mules, though practically all the infected animals were work-mules, I felt therefore that *S. calcitrans* was not transmitting the infection, because the saddle-horses would otherwise have become infected.

At this time I obtained several condemned animals, including two horses, for experimental purposes. One of the horses was placed in an isolated stable with three experimentally infected mules. In this stable the droppings were not removed, and *S. calcitrans* was permitted to breed so that the flies could be collected every day. This horse was exposed to the infected mules, and to *S. calcitrans* in this stable for 10 months, and did not become infected. At the end of this period, the horse was experimentally inoculated with trypanosomes to determine if it was susceptible or not; the animal developed the disease after an incubative period of 8 days and died 79 days after inoculation.

Furthermore, dissections of a number of specimens of *S. calcitrans* from both corrals (17 flies were taken from an experimentally infected mule) were made without ever finding a trace of a flagellate in their intestinal tracts. A few Tabanids were caught at the isolation stable, but on dissection none ever showed traces of flagellates. All attempts to incriminate biting flies, ticks, bats, or other blood-sucking vertebrates or invertebrates, failed.

Upon looking into the nature of the work the animals performed (the disease being confined to work-animals) it was ascertained that a large number of the infected animals had been in and out of the sick corral, having been usually laid up from cuts received by dirt scrapers and from collar galls. These, and other injuries, not only opened the skin, and furnished a portal of entry and exit for trypanosomes, but, as this work on scrapers is very exhausting, the animals were no doubt rendered as susceptible as possible to infection. Among the infected mules in the isolation corral it was observed that during the few days before death, excoriated patches which exuded infectious serum appeared on the neck, head and sides of the animals from their leaning against the stalls. Swarms of flies, *Musca*, *Compsomyia* and *Sarcophaga*, were attracted to these places and fed upon the exuded serum.

The impression now became confirmed that the disease was being transmitted mechanically by flies (occasionally, perhaps, by attendants) passing from infected to healthy animals, and transporting the trypanosomes which were alternately taken up and then deposited upon cutaneous wounds. Acting on this belief, it was arranged that all animals having a temperature above 101° F. should at once be isolated in a screened stable. Daily blood examinations were made of all isolated suspects, and when trypanosomes were found in a suspect, the animal was shot. Coal tar disinfectants, distasteful to flies, were freely used in this stable to keep away intruding flies that had accidentally effected an entrance, and the following recommendations were made:

The temperatures of all animals in the corrals to be taken daily, as a matter of routine, for the detection of suspects.

All animals having a temperature above 101° F. to be immediately isolated in a screened stable.

The diagnosis to be made by blood examinations; occasionally by the inoculation of mice or rats, in those cases where trypanosomes have disappeared from the peripheral blood for two or three weeks.

All infected animals to be removed as far as possible from the corrals and to be destroyed and buried.

Disinfection of halters, instruments, etc., and hands of attendants coming in contact with infected animals.

All wounds, sores, galls, cuts on well, suspected, or infected animals to be protected from flies by protective dressings containing substances such as creolin, kreso, etc. distasteful to flies.

Stray native animals are not to be impounded in or near corrals.

Sanitary inspectors and police are required to report all native and other animals suspected of having the disease.

These methods and recommendations were very largely carried out with the gratifying result that the disease, which in March, 1910, threatened to sweep through two of the largest corrals, was promptly arrested in its course and no cases have developed since May, 1910.

ON A DISEASE IN MAN FOLLOWING TICK-BITES AND OCCURRING IN LOURENÇO MARQUES.

By JOSÉ F. SANT' ANNA, M.D.[1]

Lourenço Marques.

The grass in the vicinity of the town of Lourenço Marques is often infested by larval ticks, especially in places frequented by cattle. These ticks are so numerous as to constitute a veritable scourge, since they attack a variety of animals, and man is frequently a victim to their onslaughts. The ticks are especially prevalent in July and August. Their bites may produce merely a local effect, but in some persons general symptoms may follow.

Local effects. The effects of the bites of the ticks vary. There may be simply great itching, as in scabies. In such cases little vesicles appear at the seat of the bites, and some of the vesicles are found to contain the larval ticks. These vesicles were usually noted after about eight days in the cases observed by the author, and their appearance may be accompanied by general symptoms.

General symptoms have been observed in six cases. The patient at first complains of general weakness, muscular pains, and especially of considerable difficulty in moving his arms and legs. Examined at this stage, the superficial glands in the groin and axilla are found to be acutely enlarged and painful; the glands are obviously inflamed. The enlarged glands are referable to small boils or pimples produced by some of the tick-bites; the pimples are at times very minute but nevertheless give rise to superficial lymphangitis. The glands in the neck become swollen after a short time, those situated in the nape of the neck being chiefly involved. The patient suffers from severe occipital headache, with considerable rigidity of the muscles of the nape of the neck, so that the head may be turned to one side, as in

[1] Abstract of notes supplied by the author.—G. H. F. N.

torticollis. In every case the rigidity was more marked and lasted longer in the neck than it did in the limbs. The pain and rigidity in the neck became more marked towards evening, this corresponding to an evening rise of temperature; the symptoms subsided during the night and were slight during the day-time. The pain was increased when the patient exerted himself, or laid down. The evening rise of temperature, which never exceeded 101·5° F. (38·5° C.) was preceded by chills and possessed an intermittent character.

The acute neck-symptoms began to subside after the 8th–10th day, and recovery took place spontaneously; but the glandular enlargement persisted a month or more after recovery. The glands, however, became hard and painless. The retro-auricular and mastoid glands were enlarged in all cases; the glands of one side became enlarged before those of the other side, and they appeared to stand in relation to small pimples on the scalp. The sub-maxillary glands were also enlarged, without there being any signs of pharyngitis or tonsillitis. None of the enlarged glands suppurated.

A slight papular eruption appeared on the 4th or 5th day after the tick-bites had been inflicted. The papules were not larger than a pea, and they were followed by furfuraceous scaling, without any tendency to vesiculation. None of the papules suppurated. Only in the case of a two-year-old child was a characteristic generalised eruption observed. In some cases, general weakness and muscular asthenia persisted for a time.

Treatment. Aspirine and alkalies were given internally, besides purgatives; an ointment of potassium iodide and iodine was applied externally. The drugs exerted little effect upon the course of the disease.

The ticks were nearly all *Amblyomma hebraeum* larvae, but *Rhipicephalus simus* larvae were also encountered; they were determined by Mr C. W. Howard (Government Entomologist, Lourenço Marques).

The symptoms here described have been confused locally with those of Spirochaetosis. The disease appears to have a cyclical character. Blood-smears obtained from the patients presented no pathological appearances. The affection requires further study; it is infrequent and occasionally benign, so that cases are not often observed in hospitals.

ON SYMPTOMS FOLLOWING TICK-BITES IN MAN.

By GEORGE H. F. NUTTALL, F.R.S.

The interesting Note by Dr Sant' Anna, which precedes this paper, leads me to record some observations of a somewhat similar nature to those he has reported. My information is derived from three letters received from Africa, and from Dr Hindle.

The first letter, dated 2 October, 1909, was communicated to me by Mr W. F. Cooper, B.A., having been received by him from Dr J. W. Smith. The latter, at Mr Cooper's request, made on my behalf, had written to Dr G. E. Turner, of Johannesburg, and in response to his enquiries regarding the reputed effects of bont-tick (*Amblyomma hebraeum*) bites on man, received a letter from Dr Turner in which the latter states the following:

"Certain ticks, more particularly those found on the veld of the East Coast of Natal and Cape Colony, are liable, if they bite human beings, to set up a condition which might be suitably described as a form of tick fever. The symptoms commence with a rise in temperature, headache, or a dirty tongue, and later, pain and swelling in the femoral glands. It sometimes happens that the local lesions caused by the bite of the tick are only just noticeable, and might easily be overlooked, or, if noticed, be mistaken for an ordinary flea-bite, while the general symptoms are well marked. At the end of from 24–48 hours, however, one frequently obtains an account of a kind of bleb over the bite, and some watery material being squeezed out, after which a small sore is formed. It is generally fresh arrivals to the East Coast who become infected. Dr Campbell, of Durban, tells me that persons down in that part of the country are to a great extent immune; consequently, it is

visitors and sailors who have been allowed on shore who are the most common victims. The severity of the disease varies considerably. There are generally several days during which the patient has a headache and high temperature, with painful and swollen glands, and in some cases the latter suppurate; but even where the symptoms are mild, and the glands subside without pus-formation, the effect on the general health is most depressing, it being often several weeks before the person affected feels really well. Patients of this class, if they travel, are very liable to be quarantined by the Health Authorities as Plague suspects.

It must be understood that the disease is entirely distinct from the form of tick fever produced by the bites of the large tick, *Ornithodorus moubata*, in which swelling of the glands is not a noticeable feature. I might add that on one occasion the 'Raylau Castle' came in from East London with five firemen who had temperature, dirty tongues, and enlarged glands, and the boat was put in quarantine for 24 hours pending bacteriological examination,—they were all 'tick fever'."

The second letter, dated 8 March, 1910, was written to me by Mr C. W. Howard, Government Entomologist, Lourenço Marques. It was not written with any idea of publication, but the writer has kindly consented to allow me to make use of it. Mr Howard writes:

"There have been several cases of fever contracted in Lourenço Marques recently, about which I should like to obtain your opinion. The fever always follows the bites of larval ticks which are picked up in a certain place just outside of the town where cattle are allowed to graze.

The case which first came to my notice was that of a lady, Mrs —. While out walking one Sunday, in the vicinity mentioned, she picked up a few ticks, larvae of *Boophilus annulatus decoloratus*, although at the time she gave little attention to the matter. Exactly eight days after this Mrs — came down with a very high fever. There was no clinical thermometer at hand but the fever must have gone as high as 104° C. She had to go to bed for three days, but the remainder of the time she was unable to move about much. This high fever lasted four days, disappeared for about a day and then returned for a day. The other symptoms were severe depression, violent headache, severe backache in the region of the base of the spine, an inclination to nausea, loss of appetite, and swelling in the glands of the groin on the right side, the tick-bite being on the right hip. At the same

time, the place where the tick had fastened developed into a large sore, highly inflamed and swollen, and eventually produced a core like a boil. From the first there was a small opening from which exuded a colourless liquid, but at the end pus was found for a week or more. To make sure of the diagnosis a physician was consulted, a man who has had several years' practice here. He declared it unmistakeably a tick-bite and prescribed a corrosive sublimate wash, and an ointment of Peruvian balsam and iodoform. Blood-smears showed the blood to be normal.

As I stated above, the ticks in question were larvae of *B. annulatus decoloratus*. A short time before, a herd of cattle grazing on the area where the ticks were picked up, contained several animals in which our Veterinary Surgeon found *Spirochaeta theileri*. The ticks found on these cattle include also *Rhipicephalus evertsi* and *Amblyomma hebraeum*, but *B. annulatus decoloratus* is by far the commonest, the other two being comparatively rare. Do you think there is a possibility that the *Spirochaeta* might be transmitted from the cattle to man?

At the time of her illness Mrs — had been in South Africa only 12 weeks. She had never before had fever of any sort, nor has she had it since. The infection occurred in November, a non-malarial and non-mosquito month here. She was living in a house very thoroughly mosquito-proofed and in a part of the town where *Anopheles* are extremely rare. So it cannot be considered as a case of malaria.

Some little time before this, the wife of a Transvaal official from Pretoria was visiting in Lourenço Marques, and in the same place picked up some ticks, four of which succeeded in attaching themselves. On her return to Pretoria, and in exactly eight days after picking up the ticks, she came down with fever. In her case, the fever seemed to come on alternating days and also during alternating weeks, extending in all over a month. The tick-bites produced large sores. Severe depression and extreme headache accompanied the fever. This lady also had never been exposed to mosquito bites, and her visit here was during July when no *Anopheles* were about.

Since that time I have heard of four other cases of fever following tick-bites, all of them showing similar symptoms to those of Mrs —. I am also told that in the vicinity of Grahamstown there is a place where the ticks always produce fever.

Apparently, it is only the one species of tick which produces the fever, for a few days ago Mrs — again picked up some larval ticks in a field where some goats often feed. In spite of her anxious endeavour

seven succeeded in fastening themselves, but now (fifteen days afterwards) no fever has yet developed. This time the larvae were apparently those of *Amblyomma hebraeum*[1]."

In a subsequent letter, dated 11 June, 1910, Mr Howard writes:

"You mention the fact of persons long resident in a locality becoming immune to the tick-bites. I have noticed that here. It is always visitors or new arrivals who are affected.

At the present time I myself seem to be immune. Some years ago, soon after coming to Africa, I made a journey to Northern Zululand and was there bitten by larval ticks in the grass. One bite, just below the left knee, developed into a large sore, causing severe lameness for some weeks. I, however, did not, at the time, notice fever, only slight indisposition."

Dr E. Hindle, who is at present working in the Quick Laboratory, informs me that he and Dr Breinl suffered from the effects of the bites of *Amblyomma hebraeum* larvae whilst experimenting with these ticks at the Runcorn Research Laboratories, near Liverpool. The ticks were received in 1908 from Mr C. P. Lounsbury, Government Entomologist for Cape Colony; they were infected with Heartwater and were used for experiments on goats. Whilst transferring the ticks to a goat some of them escaped and attacked the experimenters. The ticks always attached themselves about the scrotum or navel. Although they produced no local lesions, both gentlemen, after about a week, suffered from slight fever, accompanied by headache and general depression. The inguinal glands became enlarged. In one case the gland attained the size of a walnut, and it was so tender as to render walking painful. The general symptoms subsided after a few days, but the glands remained swollen for some weeks, although they were not painful after the first few days. Dr Hindle was subsequently bitten by these ticks, but there was no recurrence of the symptoms above described, this apparently indicating an acquired immunity to the effects of the bites.

That untoward effects occasionally follow the bites of Argasid and Ixodid ticks has been recorded by me elsewhere[2]; these effects are, however, not to be confused with the subsequent development of a specific

[1] The absence of any after-effects on this occasion may have been due to acquired immunity. G. H. F. N.

[2] Nuttall (1899), *Johns Hopkins Hospital Reports*, VIII. 42; (1908) *Journ. Roy. Inst. Publ. Health*, XVI. 14; (1908) *Ticks*, Part I. pp. 81 *et seq.*; (1911) *Ticks*, Part II. p. 313 (in press, see regarding the effects of *Ixodes ricinus* bites).

and recognized disease in the man or animal attacked by the tick. The Note by Dr Sant' Anna and the letters I have quoted point to a fairly definite train of symptoms following the attacks of certain ticks, and it appears justified, at least provisionally, to give to the condition the name of "*tick-bite fever*." The effects may ultimately prove to be due to either a toxin emanating from the tick or to a specific virus. We can scarcely refer to it as "tick fever," for this name has already been applied to African relapsing fever and Spotted Fever of the Rocky Mountains in man, and to the various diseases in animals due to *Spirochaeta, Piroplasma, Nuttallia* and *Theileria*. The name "tick fever" has, therefore, lost all significance, and should be dropped from our nomenclature.

AN IRON-HAEMATEIN STAIN.

WITH REMARKS ON THE GIEMSA STAIN.

By HARALD SEIDELIN, M.D.

(*From the Lister Institute, Zoological Department.*)

Plate V.

For general purposes, especially in haematological work, the Giemsa method, based on the Romanowsky principle, is certainly the most satisfactory stain for Protozoa. For certain structures, however, and in general as a control of the Giemsa stain, it is always necessary to have at one's disposal another method which will give particularly clear pictures of the nuclear elements (see *e.g.* Minchin, 1909). Of the different methods which are employed for this object the iron-haematoxylin stain is probably the most valuable and the most frequently used. There are, however, several circumstances which are unfavourable to its use as a universal stain. Its principal disadvantage is, that a differentiation is essential: the preparation is first deeply over-stained and the colour is afterwards extracted to the degree which is deemed convenient in each particular case. This extraction is always somewhat irregular, so that the observation, especially of intra-corpuscular parasites, is sometimes made very difficult, and it is not often possible to obtain in a single specimen a satisfactory stain of all the different elements. This difficulty is enhanced by the fact, that the red blood-corpuscles generally retain a deep grey, or almost black, colour. Further, a rather serious drawback is that in sections where greater masses of blood, and particularly blood-clots, are present, it is almost impossible to distinguish either intra- or extra-corpuscular parasites; to make such masses of blood

transparent it is necessary to differentiate so much, that the nuclei are also decolourized. A certain inconvenience is also experienced because of the long duration of the staining process, at least 30 hours when the original Heidenhain's method (1896) is adhered to; and although the more recent modifications, especially that of Rosenbusch (1908), have to a great extent overcome this difficulty, they still suffer from the two other disadvantages referred to.

I have therefore endeavoured to develop a quick and reliable stain of a similar nature, which does not require a differentiation. Beginning with the fact that haematein, as a rule, stains less diffusely than haematoxylin, I tried systematically mixtures of alcoholic haematein solutions and watery solutions of lithium carbonate, and obtained an excellent colouration of the tissue-nuclei, but did not find it satisfactory for Protozoa. The following formula gave the best results, staining 5 minutes:

Haematein sol. (1 % in 96 % alc.)	1 c.c.
Distilled water	4 c.c.
Saturated aqueous solution of lithium carbonate	5 drops.

Afterwards different combinations of iron-alum and haematein were tried, but always gave diffuse stains, so that a differentiation could not be dispensed with. Then I proceeded to experiment with alcoholic haematein and watery iron-perchloride solutions, after the manner of Weigert's iron-haematoxylin method (1904), which is known as an excellent nuclear stain for histological purposes, but as a rule does not stain Protozoa well. The following combinations were tried, A being a 1 per cent. solution of haematein and B the iron-perchloride solution of Weigert:

4 A + 1 B diffuse stain
3 A + 2 B good, both for sections and films
$2\frac{1}{2}$ A + $2\frac{1}{2}$ B good for sections, but too weak for films
2 A + 3 B faint
1 A + 4 B very faint.

I shall now describe the necessary details concerning the process. It is evidently only a slight modification of Weigert's method, but I believe that the difference will be found to make the present method useful in a certain number of cases, where Weigert's cannot be used.

Fixation. Sublimate-mixtures give the best results. I prefer Schaudinn's liquid: two parts of a saturated aqueous solution of mercury

bichloride and one of absolute alcohol. When this fixation is used, the pieces of tissue must be very thin, not more than 2–3 mm., but squares of about one cm. may be allowed. The tissues should be left in the fixative for 12 hours or more—two hours are sufficient for coverslip-films—but both may remain in it for over 24 hours without any bad effect. The fragments are then passed into 60 % alcohol, and subsequently into 70 % alcohol containing a small quantity of iodine, sufficient to give it a pale yellow colour; thereafter to 90 % alcohol and absolute alcohol, allowing one day in each of the four liquids. Clear thoroughly in xylol, pass to xylol paraffin then paraffin, and finally embed quickly in pure paraffin. The sections should be cut as thin as is compatible with the particular purpose in view. For trypanosomes it is generally not advisable to use sections of less than five μ in thickness. They should be fixed on clean slides after simply floating them on warm distilled water (about 40° C.) and without using any fixative such as albumen glycerin. Films are treated in the same way, only they are not brought further forward than 90 % alcohol. I have also stained formalin-fixed sections with fairly good results. Osmic vapour fixation of films has so far not proved successful, but I have not had the opportunity of trying to bleach these preparations with hydrogen peroxide previously to the staining, which might well modify the results. On the other hand, the bleaching of sections of tissue fixed in Flemming's liquid has not made it possible to obtain a good stain.

The staining process. After dissolving the paraffin in xylol the sections are passed through alcohols of diminishing strength to tap water (or distilled water). This is important, as they do not stain well when taken directly from alcohol into the stain, in spite of the latter being an alcoholic solution. The staining liquid is prepared about 15 minutes before being used, as it does not stain well immediately after mixing; it may be kept for about two hours, but will not stain after a longer period. It is prepared by thoroughly mixing three parts of solution A and two of B. The solution A is prepared by adding one gram of pure haematein (Grübler) to 100 c.cs. of 96 % alcohol, and shaking repeatedly without heating. The whole quantity is not dissolved, but the solution is preserved with the residuum and, if not completely transparent, filtered before use. The solution B is composed as follows, after Weigert: perchloride of iron 4, hydrochloric acid 1, distilled water 100. The slides are left for about five minutes in the mixture, with the sections downwards, and for this purpose there

are in use in Prof. Minchin's laboratory some very convenient glass-dishes of the exact size of a slide, so that a comparatively small quantity of the staining solution is necessary. A longer time than five minutes is only very rarely necessary, but the staining may be controlled at any moment by examining the preparation with a low power after washing, and the slide may then, if necessary, be returned to the mixture. For showing the structure of certain nuclei, for instance of lymphocytes, an even shorter time in rare cases may be preferable. The sections are washed in tap water for several minutes; a longer washing, even of several hours, does not harm them, and may sometimes be useful in making the colour blacker, if it is too blue. They are subsequently taken through alcohols of increasing strengths to absolute and then xylol, and are mounted in Canada-balsam, or Damarlack.

Results. The stain has been tried on the different materials containing Protozoa, which have been available at the present time, especially on smears of rat-blood containing *Trypanosoma lewisi*, also on the contents of the digestive tube of leeches containing trypanosomes from fresh-water fishes; most of the latter forms were very small, and many of them extremely slender and very difficult to stain by any method. The stain was further applied to many sections, *e.g.* of leeches containing the above mentioned trypanosomes, and of human spleen infected with *Leishmania donovani*; also some sections without Protozoa were treated with the stain in order to try its value as an ordinary histological method. The tests, to which the methods have been subjected, have therefore been varied, if not very numerous, and several of them have been rather severe. As a rule it may be said that the stain has given the same results as the iron-haematoxylin method, but in much less time. In some cases it has proved distinctly superior, especially in some of the sections of leeches, which contained compact masses of half digested blood; an excellent result was obtained by the present method, whilst the preparations stained after Heidenhain or Rosenbusch were quite useless, as either the whole mass presented a nearly uniform black colour, or else, when the decolouration was carried far enough to make out details, the trypanosome nuclei were decolourized also.

The karyosome and chromatin-structures stain black, the kineto-nucleus being especially dark; the flagellum is grey and shows very sharply whilst the protoplasm takes a pale grey colour. As mentioned above, instead of the proportion of 3 to 2, equal parts of the two solutions may be used in sections when a particularly strong contrast

is desirable. The protoplasm then remains nearly unstained, the structure of the tissue-nuclei is shown beautifully, and it deserves especial mention, that the limits between the cells in the several different types of epithelia are remarkably well defined.

For histological purposes a combination of this stain with the acid fuchsin—picric acid—acetic acid solution of Hansen (1898) gives far clearer pictures than the so-called van Gieson's stain as ordinarily employed. It may of course also be combined with any other counter stain, just as the ordinary haematoxylin-method, but generally with better results, as the protoplasm comes out more sharply in the colour of the counter stain, because it absorbs haematein to a much less degree than it does haematoxylin.

I consequently believe, that the staining process will not only be a useful substitute for Heidenhain's method in protozoological work, but also that it may take the place of the different haematoxylin-combinations in most cases, especially as it is both rapid and easy to carry out. However, as to its principal advantages, I may emphasize the fact that it gives a good stain in certain cases where no other haematoxylin-method can be used, and that it does away with all differentiation, a circumstance which adds greatly to the reliability of any stain. The rapid execution will be of value especially for work in tropical countries, where it is always inconvenient to keep preparations for a long time in watery solutions.

This method certainly does not compete with the Romanowsky stains, especially the Giemsa and Leishman methods, which have a field of their own in the rapid diagnosis of malaria and other blood diseases, and which have the great advantage of not producing any confusion between chromatin and pigment, between which it is sometimes difficult to distinguish in sections stained by iron-haematein, or haematoxylin. In more detailed work the use of both the Giemsa and the iron-haematein-stains will generally be necessary.

In Pl. V some figures are given, which illustrate the results obtained. The method may be summarized as follows:

I. *Sections.*

1. Fixation in sublimate-alcohol with ordinary subsequent operations, embedding in paraffin.

2. Sections 5 μ in thickness, or less. Sections to be fixed on slides without any medium.

3. Passage through xylol, and alcohols of diminishing strengths to distilled, or tap water.

4. Staining for 5–10 minutes in a mixture of 3 pts. Solution A, and 2 pts. Solution B, which has been prepared not less than 15 minutes and not more than 2 hours. Sometimes equal parts of A and B may be used.

5. Washing in tap water for 5 minutes or more.

6. Passage through alcohols of increasing strengths to absolute and then xylol.

7. Canada-balsam or Damarlack.

II. *Films.*

The only differences are, that these are not brought up to xylol after hardening, but passed from 90 % alcohol to water, and that the mixture of 3 A + 2 B is the only one that gives good results.

After the staining they are treated exactly like sections, never being allowed to dry.

Remarks on the Giemsa stain.

The Romanowsky-stain has always been very difficult to apply to sections, in all its different modifications. Leishman's method gives fairly good results, but it is a great drawback that, for obtaining them, it is always necessary to be in possession of fresh serum (1904). Also Giemsa's method was originally intended only for smears which had been dried and fixed. But fortunately it now gives, when the principles laid down by Giemsa in his later publications (1910, 1 and 2) are strictly adhered to, excellent results also after wet fixation of films, and when applied to sections. In details some slight modifications may be made, whereby still better results may, in my experience, be obtained. As the method, which should certainly prove extremely useful, strangely enough does not seem to have come into general use, I shall describe the technique as I have now been using it on different materials for a considerable length of time. In fact, I had employed the acetone-differentiation a long time before the appearance of Giemsa's papers on that subject, but I only obtained the very best results after having become acquainted with the elaborate details, with which the author himself describes the method. Therefore the reader also must be

referred to the original publications, more especially to the first of the two referred to.

Fixation. Tissues must be fixed in sublimate-alcohol, as recommended above. No other method gives, as far as I have tried, useful results, and it must be especially mentioned, that the addition of acetic acid to the sublimate-alcohol detracts from the value of the stain. When films are used, a preliminary fixation may take place in osmic acid vapours, as recommended by Minchin (1909). The following procedure is the same as that described for the iron-haematein method. It is important not to use glycerin albumen for fixing the sections on the slides.

Staining. After taking the sections through the different alcohols I have found it preferable, instead of passing them first to distilled water, to leave them in tap water until transferred to the staining solution. Sometimes it is not necessary to use the somewhat complicated process of dissolving any traces of sublimate that may remain, which Giemsa recommends. If very thin fragments of tissue are fixed the sublimate is completely taken away by the iodine-alcohol which is employed during the hardening procedures; but if only few sections are at one's disposal it may be the wiser course not to omit this precaution. A prolonged staining gives the best results. I generally use a dilution of 1 to 20 (*i.e.*, 1 drop of Giemsa's solution to each c.c. of water) for 1 hour, and then change to a dilution of 1 to 40 (1 drop to each 2 c.c.'s), in which the sections are left for about 20 hours, but sometimes I have left them for 2 or 3 days in the weaker solution and still obtained very good results. The water employed for the dilution must be distilled and have been exactly neutralized, or slightly alkalinized, after titration, with a haematoxylin solution as indicator, as Giemsa describes it. (The water must turn a faint blue in the course of from one to five minutes after the addition of one to two drops of an alcoholic haematoxylin solution to ten c.c.'s, or potassium carbonate solution must be added till that point is reached.) The water, which I have been using, has generally needed the addition to each ten c.c.'s of about two drops of a cold saturated potassium carbonate solution, but the actual proportion undoubtedly varies very much, so that a frequent titration is inevitable. An omission of this step in the technique invariably spoils the results. The slides are preferably stained with the sections downwards, using for that purpose the glass dishes mentioned above. It is also important, that both the stronger and the weaker staining solutions should be prepared immediately before being used; when left

even for a short time they lose in staining power. From the staining solution the preparations are passed to tap water, not to distilled water, and then into pure acetone, or this liquid is continually dropped on the slide until the desired differentiation is obtained. When the preparations have been strongly over-stained the decolouration may take a long time, very often half an hour or more, and then one seems to get the best results. The progress of the differentiation should be frequently controlled under the microscope. From pure acetone, which I prefer to the first mixture of Giemsa of 95 acetone and 5 xylol, the section passes into the second mixture of 70 acetone and 30 xylol, and after 5 minutes into a third mixture of 30 acetone and 70 xylol. It is decidedly better to use these three steps, instead of only two as Giemsa recommended. It sometimes happens, however, that the last named mixture of acetone and xylol becomes cloudy, but it seems to keep clear when both the xylol and the acetone are very pure; if it becomes cloudy at least equal parts of the two substances may be used. It is certainly not convenient to pass the specimens directly from 70 acetone and 30 xylol into pure xylol. After five minutes in each of the two mixtures pass into pure xylol, which should be changed once so that no trace of acetone may be left. The sections may be mounted in Canada-balsam, but I have for some time been using Damarlack, in which they should keep much longer without fading, if we can draw any conclusions from experience with other stains, which would seem to last much longer in Damarlack, as I have learned from Prof. C. J. Salomonsen in Copenhagen.

Results. This method has been tried during the last few months on the same material as the iron-haematein stain and at an earlier date on a good deal more. The different elements stain in the same way as by the ordinary Giemsa method and the results have been uniformly good on material which had been fixed in sublimate-alcohol, whilst after formalin and osmic acid they have been as constantly negative. If there is any difference, the chromatin stains still better than in dry preparations, and I can quite confirm Giemsa's assertion, that in blood smears and other films better results can be obtained after wet fixation than by the dry method.

It may be convenient to give a brief summary of this technique also, though the differences from the one given in Giemsa's paper are very small:

1. Fixation, hardening, and embedding as above.
2. Sections of a uniform thickness of 5 μ, or less.
3. Xylol, alcohols of diminishing strengths, tap water.

4. Water, to which Lugol's iodine-solution has been added in the proportion of 2 to 100, 5–10 minutes.
5. Water.
6. 0·5 % solution of sodium thiosulphate, 5–10 minutes.
7. Tap water, 5 minutes or more.
8. Diluted Giemsa-solution 1 : 20 in distilled water, which has been neutralised (haematoxylin-indicator) with potassium carbonate solution, 1 hour.
9. The same 1 : 40, 20–24 hours.
10. Wash in tap water.
11. Differentiate in pure acetone. (Control under microscope.)
12. Acetone 70, xylol 30, 5 minutes.
13. Acetone 30, xylol 70, 5 minutes.
14. xylol, 5 minutes.
15. Fresh xylol.
16. Damarlack.

I wish to express my thanks to Prof. E. A. Minchin for his kind permission to work in his laboratory, and to Dr H. M. Woodcock and Miss Muriel Robertson for material and for having tried the stain. I am also indebted to Dr J. W. W. Stephens for some material.

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Illustrating the paper by Dr H. Seidelin.
(Description: see p. 103.)

EXPLANATION OF PLATE V.

All the figures have been drawn from iron-haematein preparations with Zeiss's prism, using Zeiss's apochr. obj. 3 mm. or 2 mm. and compens. oc. 18. Figs. 1–5 × 2000, Fig. 6 × 3000. My thanks are due to Miss M. Rhodes, of the Lister Institute, for executing the drawings.

Fig. 1. *Trypanosoma lewisi* in film, using the wet method. (Fixation in Schaudinn's liquid.)

Figs. 2, 3. Fish trypanosomes from digestive tube of leech (*Hemiclepsis marginata*); wet method. (Schaudinn's liquid.)

Fig. 4. The same in the interior of the half digested mass of blood, in section of leech. (Schaudinn's liquid, paraffin section.)

Fig. 5. Part of digestive tube of the same leech. To the right are the epithelial cells; to the left the partially digested blood, and between both the trypanosomes, of which only a part of the body is to be seen, most of them having been cut through in an irregular manner.

Fig. 6. *Leishmania donovani* in section of spleen. The outline of the nucleus of the host cell has come out too darkly in the reproduction. (Formalin, paraffin.)

SOME REMARKS ON DR SWINGLE'S PAPER, "THE TRANSMISSION OF *TRYPANOSOMA LEWISI* BY RAT FLEAS," ETC.

By N. H. SWELLENGREBEL, Ph.D.
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In a recent paper (1911), Swingle describes some stages of the life-history of (1) *Trypanosoma lewisi* in *Ceratophyllus fasciatus* and *Pulex* (sp. ?), and (2) a new species of *Herpetomonas* ("*H. pattoni*") found in these fleas.

With regard to *T. lewisi*, he states that in the flea (*a*) it first becomes smaller, taking on the characteristic facies which we have called "the small trypanosome," (*b*) it then assumes the crithidia form, and (*c*) at last loses the flagellum and becomes a "cyst." To complete the life-cycle he describes small round cells, which have only one chromatin granule; they occur in the head and gut of the fleas and are regarded presumably as the infective forms of *T. lewisi*. "Conjugation" between male and female gametes is hinted at, in accordance with the present fashion of protozoologists to consider as a sexual process every accidental connection of two cells of different size in fixed and stained preparations.

In a concluding Note to the paper, Swingle states that we (1910) have described and interpreted some of his *H. pattoni* forms as part of the life-cycle of *T. lewisi*, but that our view is discredited by our having found 4 % of our control fleas infected.

We shall first offer some criticisms of his statements regarding the life-cycle of *T. lewisi*.

We would state here that in our experiments 83 fleas were allowed to feed on a rat infected with *T. lewisi* and these fleas were successively dissected on the following 18 days. During that time they were fed on uninfected rats. By this method we were able to follow all the changes in the morphology of *T. lewisi*, each stage of the development being connected to the foregoing by an uninterrupted series of forms. The life-cycle which we described was therefore not an "arrangement" of different forms, perhaps not related to one another (as Swingle seems to think possible), but represents the natural changes in the morphology of the trypanosome during its stay in the flea's gut.

A systematic research such as this was not carried out by Swingle, and in consequence he reaches the conclusions stated above regarding the life-cycle. His cycle is indeed a pure "arrangement" of the forms found by him in fleas caught haphazard on infected rats.

It was quite natural that he should find "small trypanosomes" as these represent the last stage of the development of *T. lewisi* in the flea. In such fleas small crithidiae may also be found and it was the inevitable outcome of his unsystematic method of investigation that he should think that the "small trypanosomes" changed into round forms, as we ourselves thought before having observed our complete series of fleas.

Swingle failed to observe that trypanosomes, when ingested by the flea, are first transformed into large crithidiae, and then become rounded, that these round forms produce "small crithidiae" (seen also by Swingle); afterwards these small flagellates become larger, and sometimes herpetomonad forms appear. Finally all these forms are converted into the "small trypanosomes." Swingle only found the first and last stages of this life-cycle and arranged them as well as possible.

With regard to the small round cells found in the heads of fleas, we need only say that no proof whatever is given that they belong to the life-cycle of *T. lewisi* or of any other parasite of the flea's gut.

We shall now offer some criticisms as to the identity of *H. pattoni*.

It seems that after Swingle had constructed the artificial life-cycle of *T. lewisi*, he found some of the intermediate stages of our life-cycle, and as these did not fit in with his views, it was only natural that he should create a new species for them.

Swingle did not work with fleas free from *T. lewisi*, and so he was unable to prove that *H. pattoni* is a species independent of *T. lewisi*. We think that he does not prove in the least that *H. pattoni* is

not a stage of the life-cycle of *T. lewisi*. He does not give any particulars about the methods of procuring his fleas and of hatching them out in the laboratory. He only states "the rats of Lincoln, Neb., harbour two fleas. They both contain herpetomonad forms....The flagellates found in the fleas are either pure *Herpetomonas*, pure *T. lewisi* or a mixture of the two."

We do not think that he has established the identity of his parasites, the only facts in favour of his view, that some of the flagellates of the flea's gut are natural forms (*H. pattoni*), being (1) his statement that he could not find any transitional forms between them and the true *T. lewisi*; (2) his statement that he found adult Herpetomonad forms and cysts which we had not figured in our description of *T. lewisi*; (3) the point that we had a 4% infection in our control fleas, which were supposed to be "clean" in respect to *T. lewisi*. As a matter of fact we did find forms, such as he has described as adult *Herpetomonas* and cysts, in our life-cycle of *T. lewisi*, and the infection of the control fleas might be accounted for by an old infection.

Now even if the existence of this species (*H. pattoni*) were established, this would not prove that the crithidia—and herpetomonas—stages of *T. lewisi* found by us were really natural flagellates. This is shown by the study of cultural forms of *T. lewisi* which quite resemble *Herpetomonas pattoni* (cf. Figs. 35–44 of Swingle's paper with diagram XII of ours).

His criticism to the effect that our control fleas were infected is not well founded, and moreover illogical. Even if we consider the question from his own point of view we see that only $\frac{1}{30}$ of our control fleas were infected with *H. pattoni*, whereas in his case $\frac{1}{3}$ of the fleas were naturally infected. So our fleas harboured only very few natural flagellates, whereas the forms found in fleas fed on infected rats were quite numerous, which shows that our opportunity of mixing up natural flagellates and forms of *T. lewisi* was very small. Moreover the forms we found in the control fleas all belonged to the type of small crithidiae considered by Swingle to belong to the life-cycle of *T. lewisi*.

So the only conclusion Swingle could have drawn from our control experiments was that his own conclusions were possibly wrong. We emphasised in our paper that the affinity of these small crithidiae to *T. lewisi* was not quite clear because the crithidiae were found in our control fleas. Concerning the other stages found by us (crithidiae and herpetomonads, round forms etc.) there cannot be any doubt as to their

really belonging to the life-cycle of *T. lewisi*, but these are exactly the forms concerning which Swingle has pronounced his doubts as to their systematic affinities.

To summarise, we consider that Swingle has created an artificial life-cycle for *T. lewisi* in the flea. We do not question the existence of the Herpetomonad forms which he has described, but we deny that he has established their specific identity as *H. pattoni*.

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NOTE ON THE MORPHOLOGY OF *HERPETOMONAS* AND *CRITHIDIA*, WITH SOME REMARKS ON "PHYSIOLOGICAL DEGENERATION."

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With 12 Diagrams.

CONTENTS.

I. Introduction, material and methods.

Since the publication of Prowazek's paper (1904), much work has been done on the morphology and biology of Herpetomonads and Crithidiae of the alimentary tract of several flies and other insects.

The readers of this Journal will find full accounts of the literature on this subject in the interesting papers of Miss Porter (1909), and Miss Mackinnon (1910).

During the summer of 1910 I found two species of flagellates in the gut of the blue-bottle (*Calliphora erythrocephala*), one belonging to the genus *Crithidia*, the other to the genus *Herpetomonas*. Since then Swingle (1911) described these two forms under the name of *Herpetomonas calliphorae*.

The general morphology of these flagellates being well known from the papers of Patton (1908–9), Porter, Mackinnon, Prowazek and others, I will not give a detailed description of the two parasites, but

the study of these two forms may help perhaps to gain a better knowledge of some details of the structure of these flagellates.

There is still some doubt in connection with the following points:

1. The division of the flagellum.
2. The number of flagella in non-dividing individuals of the genus *Herpetomonas*.
3. The sexual phenomena occurring in the genera *Herpetomonas* and *Crithidia*.
4. The occurrence of a true trypanosomiform-stage in the life-cycle of *Crithidia*.

Although I am quite aware of the fact that my observations do not clear these questions, they may, perhaps, help other workers to do so.

Before proceeding to the description of these flagellates, I will say a few words on the technique employed. I fixed the preparations of the contents of the gut either by the wet or by the dry method (employing corrosive alcohol and absolute alcohol respectively), and stained with iron-haematoxylin, or Giemsa's solution. Iron-haematoxylin is liable to give misleading results as some parts of the cell (*e.g.* the achromatic portion of the blepharoplast) become decolourised during the act of differentiation. Preparations stained by this method are not so trustworthy as those stained with Giemsa's solution (after wet or dry fixation).

In a previous paper (1910) I have carefully compared the relative value of the different methods of wet and dry fixation, consequently I need not dwell on this subject. I wish, however, to challenge a criticism of Jollos' (1910) concerning my paper on the technique of fixation and staining. This author states that only the structure of the nucleus is of any value if one wishes to compare the different methods of fixation. I think this view is wrong; of course the nuclear structure is very important, but in judging of the relative value of a fixative one should take into account its influence on the whole cell and not only on the nucleus. Jollos states that Giemsa himself has clearly shown the uselessness of the old method, but in reality he has only shown that one should not make dry preparations of *Amoebae*, whereas my paper refers to trypanosomes. Lastly, Jollos thinks the drawings I published to corroborate my views on the equivalent value of wet and dry fixation are not trustworthy because they are not to be compared with Minchin's figures. This is quite true, as my drawings were simple zincographic reproductions, whereas Minchin's paper (1909) is accompanied by lithographic plates; but apart from this difference, which is of no

consequence, my drawings show the same nuclear structure as Minchin's.

At times I have had some difficulty in obtaining well stained preparations with Giemsa's solution, the flagellum, especially, staining badly. In such cases I have found that good results may be obtained by immersing the preparations for some hours in serum (ordinary horse-serum preserved with a little chloroform); afterwards they are rinsed in water and stained in the ordinary way[1].

II. *Crithidia calliphorae* n. sp.

This species was only found once and so I cannot give a description of the whole life-history. It is certainly different from the *Herpetomonas* also found in the gut of *Calliphora erythrocephala*, but it is possible that this species is identical with *C. muscae-domesticae* (Werner), as described by Rosenbusch (1909).

The full-grown organism is 12–21 μ long (without the flagellum) and 2 μ broad; the whole flagellum is 19–31 μ long, the extracellular part of it measures 15–28 μ. It looks rather like a *Herpetomonas*

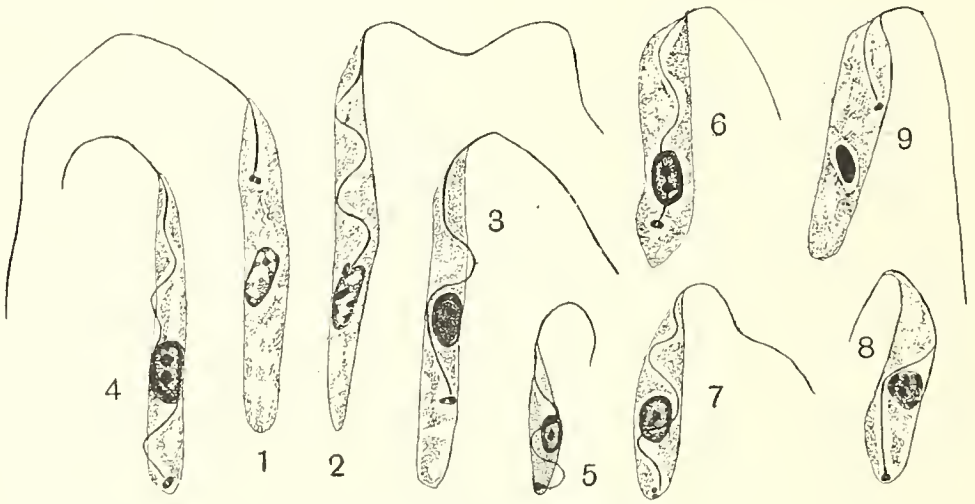

Diagram I. *Crithidia calliphorae.*

1. Herpetomonas-form.
2. Crithidia-form.
3–8. Trypanosome-forms.
9. Herpetomonas-form (wet fixation, iron-haematoxylin).

Nearly all the samples of Giemsa solution sent to me by Messrs Grübler and Holborn of Leipzig have given excellent results, and I desire to thank the Firm for the amiable way in which they helped me in the matter of stains.

(Diagram I, Fig. 1), but often a distinct undulating membrane is present (Fig. 2).

The blepharoplast may be situated near the anterior part of the flagellate (Fig. 1), or in the neighbourhood of the nucleus (Fig. 2); or it may even pass the nucleus and come to lie in the posterior part of the cell (Figs. 3–8), so that a real trypanosome-facies is produced.

Judging from the numerous intermediate stages between the herpetomonas- and trypanosome-forms, it is obvious that these trypanosomes do not arise by the flagellum bending back along the body of the flagellate, as in Mackinnon's *Herpetomonas* of *Homalomyia*. Trypanosome-like flagellates of non-bloodsucking insects have been found by Chatton and Alilaire (1908), Roubaud (1908), and Swingle (1911); Roubaud found transitional stages between the herpetomonas- and trypanosome-facies. Woodcock (1910) thinks these forms are not real trypanosomes, because an undulating membrane does not exist, the

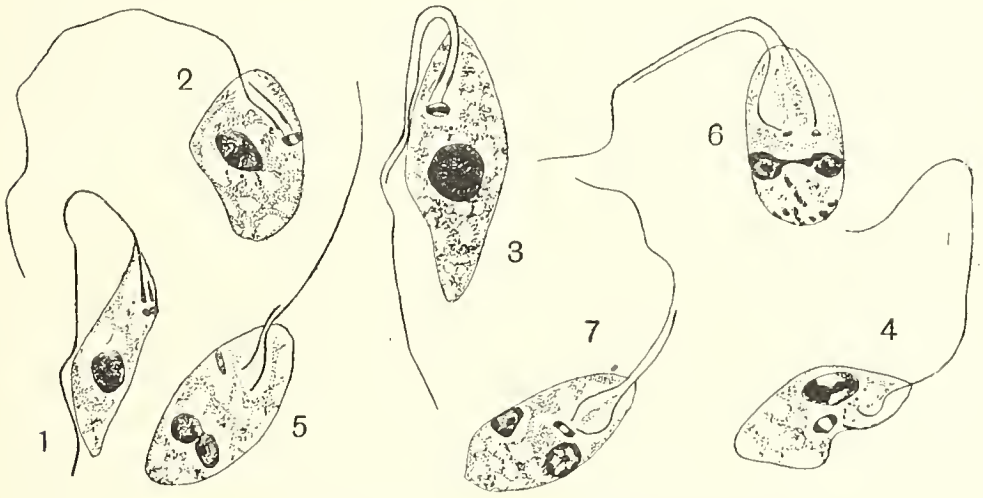

Diagram II. Stages of division of *Crithidia calliphorae*.

1. Formation of a new flagellar root.
2–3. Division of the blepharoplast; growing of the new flagellum.
4–8. Nuclear division.

flagellum passing through the cell and not alongside it. This is indeed the case with Roubaud's flagellate, but in *C. calliphorae* I think there can be little doubt that a real undulating membrane occurs in the trypanosome-facies. These forms are 10–16 μ long and 2–2·3 μ broad. The whole flagellum is 15–23 μ long; the free flagellum 4·5–9 μ.

As in *Trypanosoma lewisi* the blepharoplast is composed of an achromatic body in which one or two chromatic granules are embedded.

The nucleus contains a peripheral chromatic area and one or more central granules. During the cellular division (Diagram II, Figs. 1–8) the flagellate becomes shorter and broader, the blepharoplast becomes spindle-shaped (Figs. 2, 3) and the nucleus larger (Figs. 1–3). The nuclear division seems to be a promitosis, the karyosome becoming elongated (Fig. 4) and dividing (Fig. 5). After division, the two daughter-nuclei remain united for some time by a chromatic filament (Fig. 6). The formation of the new flagellum proceeds in the same way as I described (1910) in *Trypanosoma lewisi*. A short flagellum (flagellar root) is produced by the base of the old flagellum and grows out independently (Figs. 1–3, 5, 6). The dividing forms are 9–13 μ long, and 5–6 μ broad.

In never-dried preparations, fixed with corrosive alcohol and stained with iron-haematoxylin (Diagram I, Fig. 9), the blepharoplast does not show any structure, owing to the fact that the achromatic portion of this organellum is left uncoloured. The nucleus stains deeply, without any differentiation, and is surrounded by a clear halo, due to the contraction of the nuclear substance. Similar figures have been mistaken for a nucleus containing a large karyosome, and clearly illustrate how defective is this method of fixing and staining.

III. *Herpetomonas calliphorae* Swingle.

This species has been described recently by Swingle (1911). In his description *H. calliphorae* and *Crithidia calliphorae* are thrown together. There can be little doubt, however, that they are distinct species, as the morphology and the mode of division are quite different.

The prevalence of this parasite in the flies varies according to the time of the year. In August (in Amsterdam) 24 % of the flies caught were found infected; in October, 50 % were infected: in November, 24 %. The few flies caught during the time from December till March were apparently not infected, but a few post-flagellate stages may have been overlooked.

1. *Flagellate stage.*

The non-dividing flagellate is 10–25 μ long (without flagellum) and 2–3 μ broad; the whole flagellum is 43–45 μ long, the extracellular part of it 39–40 μ. Sometimes very slender forms are to be found (Diagram III, Fig. 3) measuring 23 μ in length and only 1·2 μ in

breadth. The nucleus is of the ordinary structure: peripheral chromatin, and one or more central chromatic bodies (Diagram III, Fig. 1). The blepharoplast is very large (1·8 μ long and 0·6–1·2 μ broad) as in other herpetomonads, but the peculiarity of the blepharoplast of this flagellate is, that it shows a rather complicated internal structure, which very much resembles a real nucleus. It is composed of a peripheral chromatic layer surrounding an achromatic substance, in which one or more chromatic granules may be seen. This large

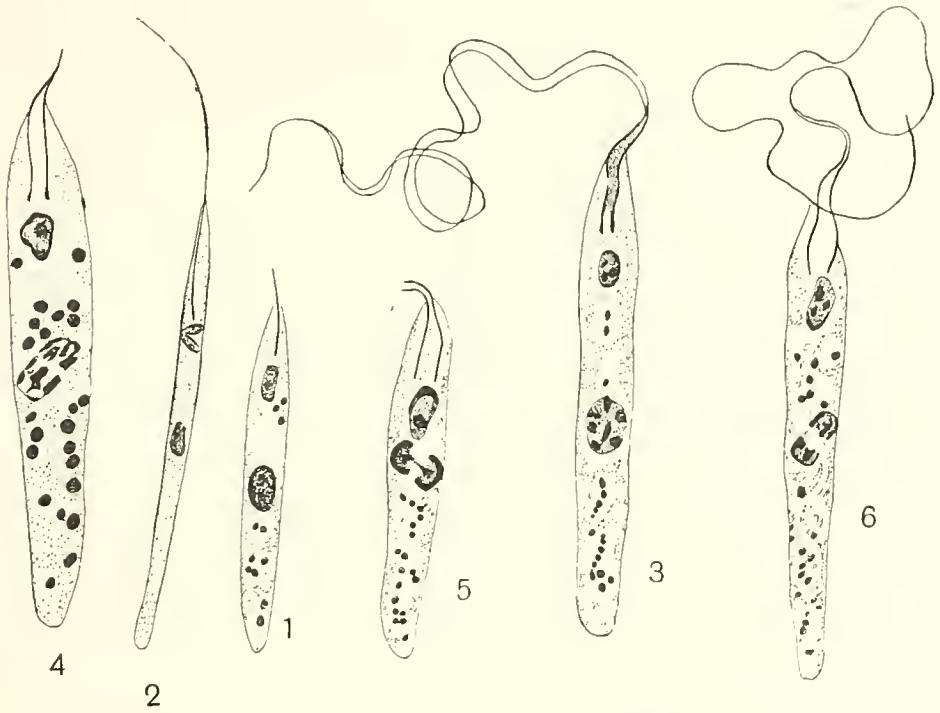

Diagram III. *Herpetomonas calliphorae.*

1. Normal form. 2. Slender form.
3. Blepharoplast become enlarged. Membrane between the two flagella.
4–6. Division of the nucleus; in Fig. 6 an axial uncoloured filament is present; in Fig. 4, beginning of the division of the blepharoplast.

blepharoplast might be regarded as an artefact due to Giemsa's stain "exaggerating" the chromatin (according to Minchin), but this view is untenable, because preparations fixed and stained in accordance with recent methods (corrosive alcohol, iron-haematoxylin) show blepharoplasts of the same size (Diagram VI, Figs. 1, 2). The protoplasm often contains chromatin-like granules, which do not become decolourised, when treated with 1 % sulphuric acid, and which are blackened with

iodine after staining with Giemsa's solution, so these granules consist of volutin, a substance found already in trypanosomes and other Protozoa (Swellengrebel, 1910; Hindle, 1910). I have never seen the axial filament described by Prowazek, but sometimes an uncoloured spirally-wound line may be seen passing through the cell (Diagram III, Fig. 6). Similar structures have been mentioned by Miss Porter.

The base of the flagellum is a very complicated structure (Diagram IV, Fig. 7). Often a distinct basal granule is to be seen and another thickening of the flagellum is present at the point where it becomes free. This latter granule has been described by Berliner (1909); I propose to call it the "marginal granule." Between these

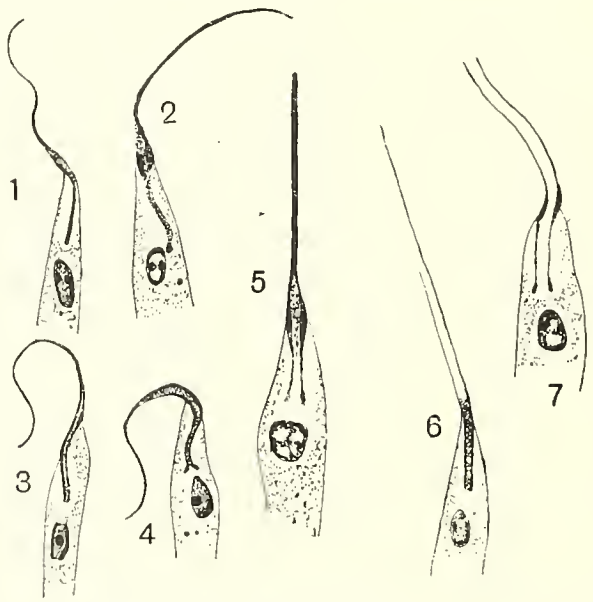

Diagram IV. Production of a new flagellum in *Herpetomonas calliphorae*.

1–2. Fission of the marginal granule; in Fig. 2 the basal granule is distinct.
3. Fission continues along the rhizoplast.
4–5. The basal granule is also divided.
6. This figure suggests that the new external flagellum is not produced by fission of the old one, but by independent growth.
7. Two new flagella are produced each showing distinct basal and marginal granules.

two granules (basal and marginal) is situated that part of the flagellum, which is generally called "rhizoplast"; it stains pinkish with Giemsa, whereas the rest of the flagellum stains crimson.

Herpetomonads are frequently found which possess two flagella without showing any sign of division; as, however, in other flies similar flagellates may be found with only one flagellum, I think Patton

(1908–9) is right in asserting that the possession of this double flagellum is not a generic character of *Herpetomonas*. Still there is some difficulty with respect to this matter: Patton and his co-workers regard the presence of the double flagellum as an early sign of division, but I sometimes found that the percentage of flagellates showing undoubted signs of division (of the nucleus or the blepharoplast) was larger among herpetomonads with one flagellum than among herpetomonads with two flagella. Of 400 herpetomonads with only one flagellum, 23 showed two nuclei and 377 did not divide; on the other hand, 400 herpetomonads with two flagella did not show any other sign of division. Perhaps the possession of a double flagellum is a sign of division proceeding under unfavourable circumstances.

The statements of different authors are not in agreement regarding the division of the flagellum: some assert that division results from longitudinal fission (Porter), others maintain that a new flagellum arises at the base of the old one (Berliner, Mackinnon). I have carefully studied this subject and have come to the following conclusions (Diagram IV). The first stage of the production of a new flagellum is represented in Diagram IV, Figs. 1–2. The marginal granule becomes larger and divides into two chromatic granules, united by an achromatic substance; one of these granules is directly connected with the old flagellum.

The achromatic substance seems to be the continuation of the rhizoplast, and like this, it stains pinkish with Giemsa. In the next stages (Figs. 3–5) the rhizoplast shows longitudinal fission and the basal granule divides. There are now two rhizoplasts, one connected with the old flagellum, the other with the free marginal granule (Figs. 4–5).

I am not sure whether the fission of the flagellum proceeds also in a direction opposite to that of the rhizoplast, because the two flagella remain entangled even when fully grown out. Some observations (Fig. 6) seem to prove, however, that the new flagellum becomes emancipated very early, and grows independently of the old one. Berliner has made a similar observation. The two flagella remain connected for a long time at the base by the achromatic substance already described (Diagram III, Fig. 3; Diagram IV, Fig. 6), which forms the delicate interflagellar membrane, mentioned by Prowazek; its presence accounts for the fact that sometimes the two flagella may remain entangled even when the two daughter-cells are nearly separated.

The nuclear division proceeds in the ordinary way, the central chromatic granule dividing first (Diagram III, Fig. 4), the rest of the nucleus afterwards (Figs. 5, 6).

The division of the blepharoplast is interesting and deserves a little more attention (Diagrams III and V). During cellular division the blepharoplast becomes larger and the chromatic granules are often multiplied (Diagram III, Figs. 3, 5, 6). Then longitudinal division begins (Diagram III, Fig. 4; Diagram V, Figs. 1–6); the blepharoplast

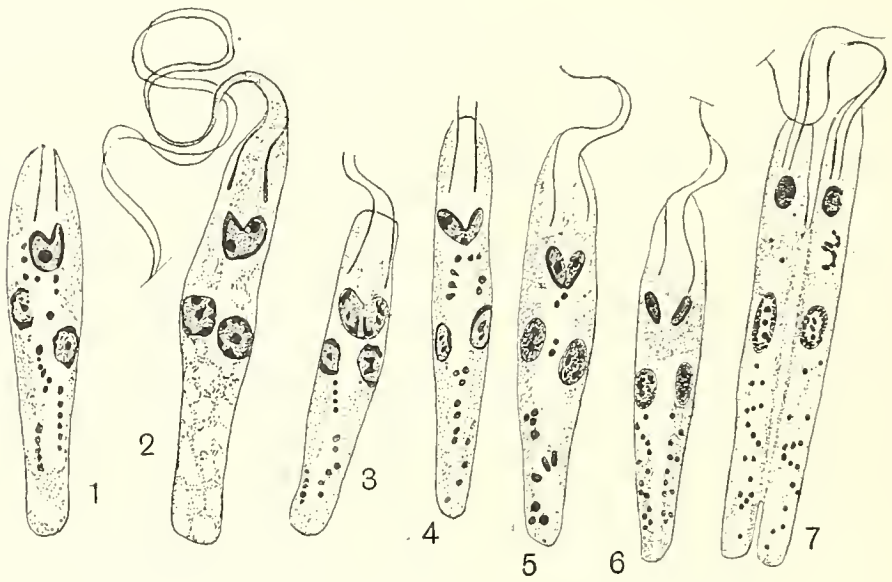

Diagram V. Cellular division in *Herpetomonas calliphorae*.

1–6. Division of the blepharoplast.

7. Nearly completed cellular division.

becomes V-shaped, the two daughter-blepharoplasts forming the arms of the V, which afterwards become separated from each other. If the granules in the blepharoplast are divided, the two daughter-blepharoplasts generally receive each a part of the chromatic substance (Diagram V, Figs. 2, 3, 5). But often the distribution of the chromatin is not so regular, so that one blepharoplast holds all the granules and the other none (Diagram V, Figs. 1, 4–6). Afterwards all the blepharoplasts in the dividing cells are found to contain granules; it might therefore be assumed that the latter can be regenerated. One might also be tempted to compare these granules in the blepharoplasts with nuclear karyosomes, but their irregular behaviour renders such a comparison doubtful.

In preparations stained with iron-haematoxylin these changes in the structure are very indistinct; Diagram VI (Fig. 3) shows that the figures are, in general outline, the same as after Giemsa's stain.

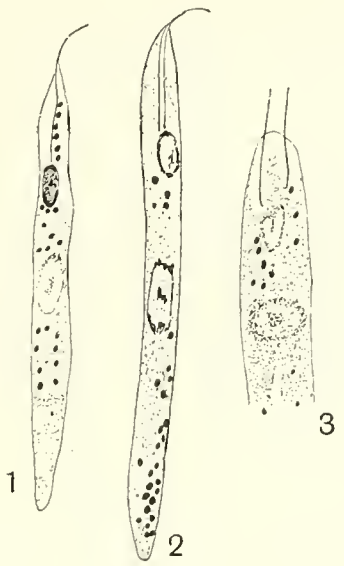

Diagram VI.

Herpetomonas calliphorae after fixation in corrosive-alcohol and staining with iron-haematoxylin. Fig. 3 shows a stage of division (V-shaped blepharoplast).

2. *Preflagellate stages.*

These are round cells measuring 7–10 μ in length, and 6–9 μ in breadth (Diagram VII). They are to be found in the crop. The nucleus has sometimes a distinct achromatic karyosome staining blue with Giemsa's solution. The blepharoplast is generally large, the flagellum is either completely absent or a pink-staining rhizoplast, single or double (Figs. 1–3), may be seen near the blepharoplast; sometimes a distinct short flagellum is present (Figs. 6, 7). Division is common among the preflagellate stages as already pointed out by Patton (Figs. 3, 4). Fig. 4 shows stages of division of the nucleus and blepharoplast. The nuclear division is of the common pattern; the distribution of the chromatin in the dividing blepharoplast is rather irregular; in Fig. 4 it suggests a sort of mitotic spindle, but I presume it is only accidental and has no special significance.

Large chromatoid granules, composed of volutin, are conspicuous in the preflagellate stages, and are much more numerous than in the

flagellate stages. Sometimes it is difficult to recognise the blepharoplast among the volutin granules, because they both stain in the same way (dark crimson with Giemsa, deep black with iron-haematoxylin stain)[1]. In some cases I found anuclear preflagellate forms, but the presence of the volutin granules gives them a multinuclear appearance (Fig. 5); such cells closely resemble some stages of development considered by Prowazek as the end of an "etheogenesis" (see below).

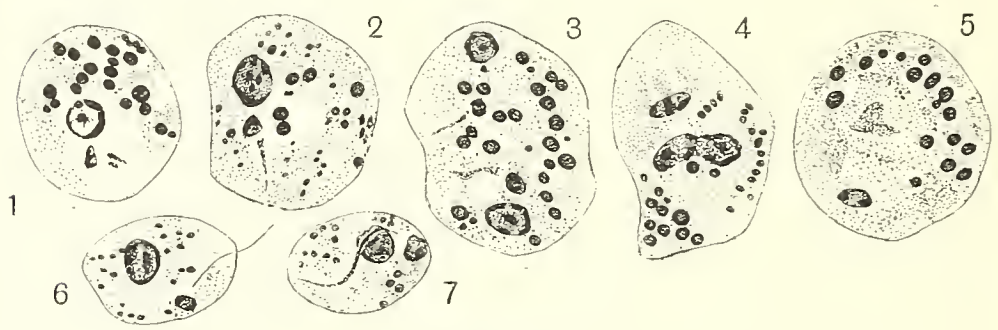

Diagram VII.

Preflagellate stages of *Herpetomonas calliphorae* in the crop of the fly.

1–2. Non-dividing forms; the nuclear karyosome shown in Fig. 1 was stained blue with Giemsa's stain.

3–4. Dividing forms; in Fig. 4 a pseudo-mitotic division of the blepharoplast.

5. Degenerated preflagellate; the nucleus has disappeared.

6–7. Forms with a short external flagellum.

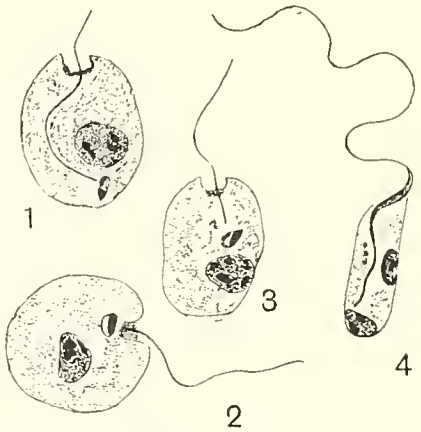

Diagram VIII.

1–3. Preflagellate forms of *Herpetomonas calliphorae* in the midgut.

4. Transitional stage towards the formation of a full-grown *Herpetomonas*.

[1] Recently, blepharoplast-like inclusions have been found in some Hematozoa (*Halteridium*). It would be worth while to ascertain if these inclusions are real blepharoplasts, chromidia, or simply volutin-granules.

By subsequent division, large rosettes of preflagellate forms may be produced, such as Patton describes.

Preflagellate stages may persist for a long time, not only in the crop, but also in the midgut (Diagram VIII, Figs. 1–3); they possess a short flagellum and move about actively. In some flies the midgut only contains preflagellates; in such cases, the latter might be mistaken for an independent species. The preflagellates show a peculiar invagination of the ectoplasm, just where the flagellum becomes free; this invagination takes a pinkish hue with Giemsa, proving that it is of ectoplasmic origin; it forms a short canal through which runs the flagellum. I suppose that this invagination may be regarded as a rudimentary cytostome, such as may be found in a complete state in free-living flagellates.

The preflagellate forms develop into real herpetomonads. Fig. 4 shows a transitional stage in this part of the life-cycle.

3. *Post-flagellate stages.*

When the post-flagellate stages are formed in the rectum, the flagellate becomes smaller (Diagram IX, Fig. 1); the blepharoplast

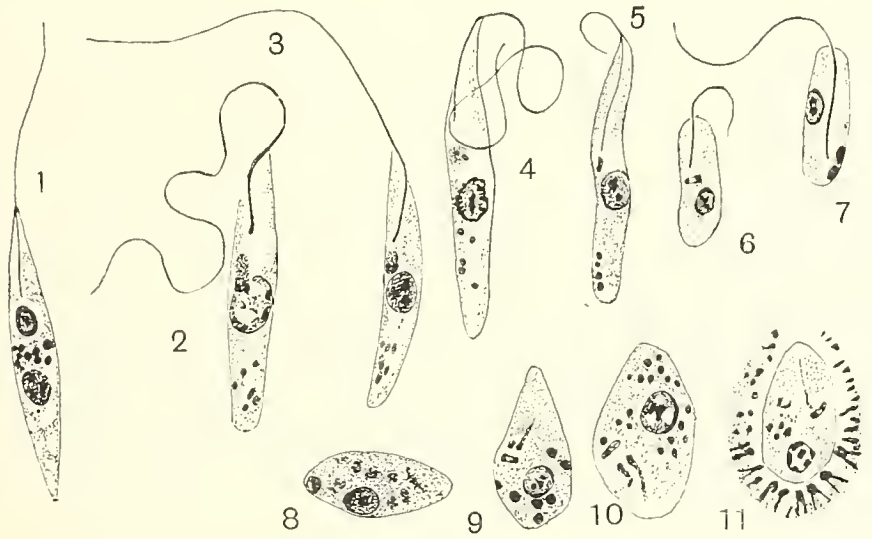

Diagram IX.

Formation of the post-flagellate stages of *Herpetomonas calliphorae*.

1. Normal flagellate becoming smaller with short flagellum.
2–5. Elimination of the achromatic part of the blepharoplast.
6–7. Last stages before the production of the "cysts."
8–10. "Cysts" without a cyst-wall; Fig. 9 shows a "cyst" with double rhizoplast.
11. "Cyst" with a "cyst-wall."

loses a part of its achromatic substance and consequently becomes much smaller (Figs. 2–5). The flagellate finally becomes very short with only an internal flagellum, or no flagellum (Figs. 6–11). Sometimes a double rhizoplast may be seen (Figs. 9, 10). These forms cannot be considered as real cysts as a well-developed cyst-wall was not observed.

Sometimes a peculiar radiated zone was seen surrounding the cell and staining pinkish with Giemsa (Fig. 11). Similar observations were made by Minchin (1908) and Prowazek (1904); the latter considers this formation a cyst-wall. I think these radiated zones do not really exist but are artefacts due to the precipitation of staining substance around the cell, as may be often observed when Giemsa solution comes in contact with gelatinous substances, but after all these artefacts may be an indication that there is a gelatinous envelope present.

The post-flagellate stages are 7–9 μ long and 4–5 μ broad, the gelatinous envelope is 1·5–1·8 μ broad.

In the same way as the preflagellate stages, the post-flagellate stages may be found in the midgut, often without any other developmental form of this parasite (Diagram X). They are small flagellates

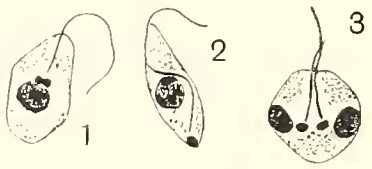

Diagram X.

Transitional forms between the flagellate and post-flagellate stages of *Herpetomonas calliphorae*, from the midgut of the fly.

with a short flagellum and are actively motile, often uniting into large clusters. Sometimes stages of division are to be seen (Fig. 3). Similar forms occurring in the house-fly (*Musca domestica*) have been probably mistaken by Werner (1908) for an independent species (*Crithidia muscae-domesticae*).

4. *Degenerating forms.*

These forms (Diagram XI) are frequent in flies which have been fed on fruit-juice or on honey, especially in autumn. Sometimes the nucleus degenerates (Fig. 6) so that only the blepharoplast remains (Fig. 5); or the nucleus and the blepharoplast may both be reduced to

chromatic granules scattered about the protoplasm (Fig. 1). Similar forms have been regarded as male gametes, of course without any proof.

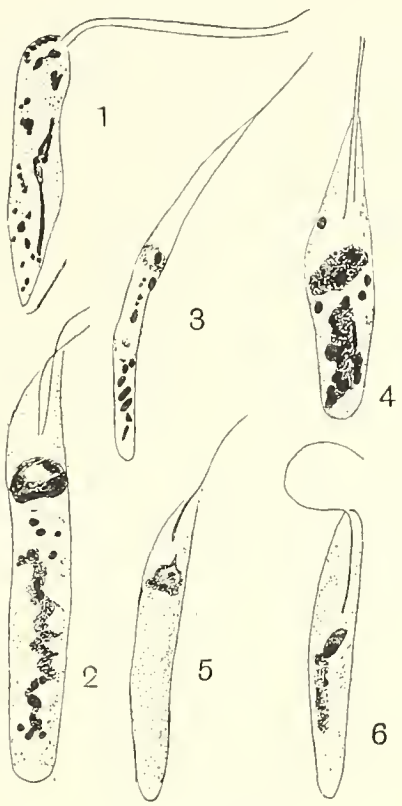

Diagram XI.

Degenerative changes of *Herpetomonas calliphorae* (flagellate form), in the midgut of a fly, fed on honey.

1. "Chromidial" flagellate.
2, 4. Hypertrophy and desintegration of the nucleus and the blepharoplast.
3. Flagellate stained with Giemsa's solution and treated afterwards with iodine to show the distribution of volutin. Note the volutin granules in the blepharoplast.
5, 6. Anuclear flagellates.

Especially interesting are the flagellates represented in Figs. 2–4, from a fly which had been fed on honey for one day. All these flagellates had become immobilised and showed a granular protoplasm. In stained preparations the nucleus and the blepharoplast showed hypertrophy (Fig. 4); and sometimes the nucleus was elongated into a spirally-wound filament (Fig. 2). Numerous granules were scattered about the protoplasm, and were composed of volutin. Fig. 3 (showing a flagellate treated with iodine after Giemsa's stain, to make the

volutin granules conspicuous) suggests that volutin may be produced by the blepharoplast.

In the rectum of some flies flagellates were found, which were obviously checked in the act of becoming post-flagellate forms (Diagram XII), because no complete post-flagellate stages were found. These forms showed peculiar changes of the normal structure such as hypertrophy of the nucleus and the blepharoplast (Figs. 1, 2), or premature division of these organella (Figs. 3, 4, 7).

Sometimes the chromatic part of the blepharoplast was seen to divide and the surrounding achromatic substance was expelled, so that only two large chromatic bodies remained (Figs. 5–6).

Some of the developmental forms of *Herpetomonas muscae-domesticae*, described by Prowazek (1904) as stages of "parthenogenesis" and "etheogenesis," bear a remarkable resemblance to these abnormal flagellates figured in Diagram XII.

In Prowazek's "etheogenesis," the "male" forms are stated to exhibit a degeneration of the nucleus, the blepharoplast is divided into four parts, the two pairs of blepharoplasts copulate and at last large plasmatic globules are produced, containing several blepharoplasts.

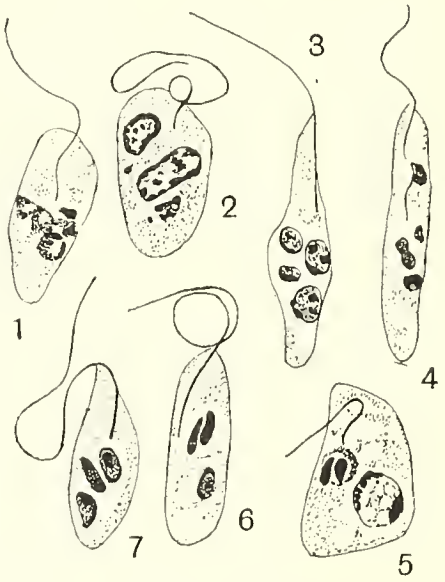

Diagram XII.

Degenerative changes of *Herpetomonas calliphorae* in the rectum.

1. Hypertrophy of the nucleus.
2–4, 7. Premature division of the nucleus and the blepharoplast.
5–6. Hypertrophy of the blepharoplast. Elimination of achromatic substance.

This last stage resembles the degenerating preflagellate form represented in Diagram VII (Fig. 5).

It may be noted that Prowazek does not mention the preflagellate stages in his construction of the life-cycle of *Herpetomonas muscae-domesticae* and so a confusion with these stages was quite possible.

During the "parthenogenesis" the nucleus is divided into two large and two small nuclei, just as in Diagram XII, Fig. 3. The result of this development is the formation of small non-flagellate cells each containing a nucleus and a blepharoplast. According to Patton's nomenclature we should call these stages simply post-flagellate forms.

It seems probable that Prowazek in his "parthenogenetic" and "etheogenetic" cycle has put together forms which have no close relation to each other. Until further confirmation is forthcoming these cycles should be regarded with some suspicion.

IV. General Remarks.

1. *Similarity in structure of some Bacteria and degenerating Flagellates.*

Stages of degeneration such as are figured in Diagram XI (Figs. 1, 2, 4), where the nucleus becomes diffuse (often forming a chromatic band wound in a more or less distinct zig-zag line) and a general lack of differentiation of the internal cellular structure may be noted (Fig. 1), seem to be frequent among trypanosome-like flagellates. Similar changes were noted in *Trypanosoma lewisi* in the blood of the rat (Swellengrebel, 1910), and in the gut of different invertebrate hosts (Swellengrebel and Strickland, 1910).

Robertson (1906) has noted a zig-zag line of chromatoid substance (volutin) passing through the body of *Trypanosoma brucei*, but here the nucleus remains unaltered and only volutin-granules are expelled from it. Robertson has already drawn attention to the analogy of these zig-zag structures of *Trypanosoma* and similar chromatoid filaments in *Spirochaetae* (Perrin, 1906; Swellengrebel, 1907; Fantham, 1908) and bacteria (Swellengrebel, 1906–1909; Dobell, 1908–1909; Guilliermond 1909; Mencl, 1911, etc.). The diffuse nuclei of *T. lewisi* and *Herpetomonas calliphorae* bear a remarkable resemblance to the chromatin-spirals of many bacteria (*e.g. Bacillus flexilis*; *B. lunula* of Dobell; *Sphaerotilus natans* of Swellengrebel) much more than the above mentioned chromatoid filaments of *T. brucei*. The value of this analogy

in structure between bacteria and flagellates is only relative because it refers to an abnormal structure of the flagellate compared with a normal structure of bacteria. I nevertheless believe that this similarity in structure may indicate a more than superficial relation between some flagellates and bacteria; Schaudinn (1902–1903) has already expressed the opinion that such a relation exists. It would however be premature to base any far-reaching speculations on these isolated observations; I have mentioned them here only to draw attention to this interesting systematic question of the supposed mutual affinities of bacteria and flagellates.

2. *Degenerative appearance of some protozoa in relation to Hertwig's "physiological degeneration."*

In the last two years much work has been done by the contributors to this Journal on the phenomena occurring during the degeneration of some Haematozoa, *e.g. Trypanosoma* and *Piroplasma*. This work was originally undertaken with the object of clearing up certain errors in the interpretation of so-called sexual processes which had been described in the life-history of these organisms, but I think the facts now collected may serve as a base for a broader view.

In 1882 Weissmann formulated his well-known theory on the immortality of unicellular organisms. The individual may divide and disappear in that way, also parts of the living cell may be thrown off or become destroyed, but death, *i.e.* destruction of the whole organism in another way than by formation of two or more daughter individuals, does not occur under normal (physiological) conditions.

This theory was seriously contested by Hertwig (1904) who thinks that "physiological death," *i.e.* death originated by the vital functions themselves, occurs among unicellular organisms and so may be considered to be an intrinsic attribute of life. Only cells with reduced vital functions may live for an indefinite time (gametocytes).

The cause of this physiological death is an abnormal relation between the nucleus and the protoplasm, leading to the so-called "physiological degeneration," and at last to death. Among unicellular organisms it may be prevented by encystment or conjugation, both processes leading to a reduction of the nuclear apparatus and thus re-establishing the normal relation between protoplasm and nucleus.

If these regulating influences do not come into action, the "physiological degeneration" begins. It is characterised by the hypertrophy

of the nucleus (especially of the nucleoli), by premature division of the nuclei, and by elimination of nucleolar substance into the protoplasm. Finally the nuclei are completely destroyed ("chromidial animal") and death can no longer be avoided. Hertwig calls these phenomena "physiological" because he thinks they are the natural consequences of vital functions and can only be avoided by special means (conjugation or encystment).

The degenerative changes seen in some Trypanosomes (*T. lewisi, gambiense, brucei*), in *Piroplasma canis*, and in *Herpetomonas calliphorae*, seem to be of the same nature as those seen during physiological degeneration of *Actinosphaerium eichhorni* (a Heliozoon studied by Hertwig).

In *Piroplasma canis*, Nuttall (1910) observed elimination of chromatic substance under the influence of trypanblue. In *Trypanosoma brucei* chromatoid granules are thrown out from the nucleus under the influence of arsenophenylglycin (Nuttall, 1910).

In *Trypanosoma gambiense*, masses of chromatic substance are produced in the nucleus and are thrown out into the protoplasm as volutin granules. Under unfavourable circumstances (death of the host, arsenophenylglycin) there is a hyperproduction of volutin ("volutinosis," Hindle, 1910; Swellengrebel, 1909). In this case there is a striking resemblance to the degeneration of *Actinosphaerium*; in both cases a definite part of the nucleus becomes hypertrophied (nucleoli in *Actinosphaerium*, chromatic masses in *Trypanosoma*) and is expelled into the protoplasm, where its chemical structure is altered: the chromatin is changed into volutin in *Trypanosoma*, and into pigment-like substance in *Actinosphaerium*. In analogy with observations on *Actinosphaerium* we note that these nuclear changes of *T. gambiense* coincide with the cessation of the power of division.

In *Trypanosoma lewisi* we observe under abnormal conditions (preservation in the refrigerator for a long time) hypertrophy of the nucleus and the blepharoplast. Here again it is a special part of the nucleus (karyosome) which grows at the cost of the remaining nuclear substance and is afterwards thrown out into the protoplasm, in the form of irregular filaments (Swellengrebel, 1910).

Herpetomonas calliphorae shows similar changes. We note hypertrophy of the nucleus and the blepharoplast, especially of the karyosomatic substance, at the cost of the remaining nuclear material, elimination of this substance in the form of volutin granules, and

complete cessation of nuclear division. In this case we not only find hypertrophy of the nuclear apparatus, but also premature division unaccompanied by hyperproduction of volutin. So an antagonistic relation seems to exist between the power of nuclear division and the hyperproduction of volutin, which is in accordance with Hertwig's observations on *Actinosphaerium.*

We note a remarkable analogy between the degenerative appearances of the above mentioned flagellates and of *Actinosphaerium*, but here I think the analogy ends, for it is impossible to consider the degeneration of *Trypanosoma* and *Herpetomonas* as "physiological," *i.e.* as a necessary consequence of vital functions, only to be avoided by copulation or encystment. It is true that in the case of *Herpetomonas* encystment occurs; it is even accompanied by elimination of chromatic substance from the blepharoplast; but even here no degeneration occurs unless unfavourable trophic conditions occur (honey, fruit-juice, provoking the development of yeast cells and bacteria, which produce toxic substances).

Trypanosomes remain apparently healthy for an unlimited number of generations; the degenerative phenomena already described occur only when distinct toxic influences (death of the host, drug treatment, etc.) come into action. It is true that cyclical depressions and revivals have been described recently in *Trypanosoma gambiense* (Ross and Thomson, 1911) and Fantham (1911) has shown that these depressions coincide with the production of resistant forms; but according to Ross and Thomson these depressions occur under the influence of protecting reactions from the body of the host. The degeneration of *Piroplasma canis* is also due to toxic effects. So we see that in all these cases the degeneration is not "physiological" but directly due to external noxious influences. If these influences are checked no such degeneration takes place.

One may ask whether this is a special condition of these parasites or whether the "physiological" degeneration of Hertwig actually exists. I cannot answer this question, but the researches referred to suggest the possibility that "physiological" degeneration of *Actinosphaerium* and "depression" in cultures of infusoria, as observed by Calkins (1909), are due to toxic substances produced in these cultures and are not a consequence of the vital functions, *eo ipso.* Similar doubts have been recently expressed by Borowsky (1910).

V. Summary.

1. In accordance with the views held by Chatton and Alilaire (1908) and by Roubaud (1909), I have seen that a true trypanosome-stage occurs in the life-history of *Crithidia calliphorae*. These trypanosomes have an undulating membrane and cannot be considered as herpetomonads with their blepharoplasts situated behind the nucleus and a completely internal flagellum (Woodcock), nor are they herpetomonads with the flagellum bent back along the body of the cell (Mackinnon). This existence of a trypanosome stage in the life-history of *Crithidia* is quite comprehensible if we consider that in many cases true *Trypanosomata* are changed into *Crithidiae* when they pass from the blood into the gut of an invertebrate host (*T. gambiense, T. lewisi, Schizotrypanum cruzi*, etc.).

2. There is no sharp difference between the genera *Crithidia* and *Herpetomonas*. In *Crithidia calliphorae* forms may be observed without any trace of an undulating membrane (Diagram I, Fig. 1); in *Herpetomonas calliphorae* such an organellum may be sometimes present (Diagram IX, Fig. 4).

Still I think that it is not permissible to throw these two genera together, as, generally speaking, an undulating membrane is present in *Crithidia* and absent in *Herpetomonas*.

3. *Herpetomonas* is often biflagellate, even when no signs of cellular division are present and the flagellates multiply less actively than in monoflagellate stocks. Still I agree with Patton that this biflagellate condition is too inconstant to permit of its being recognized as of generic significance.

4. The structure of the blepharoplast of *Herpetomonas* is distinctly like that of a nucleus, with peripheral chromatin and one or more central granules. It is true that the behaviour of these granules during the division is not so regular as that of the karyosomes of real nuclei, nevertheless, I think that the aspect of the blepharoplast of *H. calliphorae* is a strong argument in favour of Schaudinn's, and also Minchin's, view that this organellum must be regarded as a specialized nucleus (kinetonucleus).

5. The flagellum of *Crithidia calliphorae* is constructed in the same way as that of *Trypanosoma lewisi*: a basal granule is present and the new flagellum is formed by the production of a new flagellar root by fission of the basal part of the old flagellum. In *Herpetomonas*

calliphorae a basal granule (at the base of the flagellum) and a marginal granule (at the point where the flagellum reaches the surface of the cell and becomes free) are present. The part of the flagellum situated between these two granules is the rhizoplast. The marginal granule first divides; then follows the fission of the rhizoplast and basal granule; lastly, from the marginal granule a new flagellum grows out, so closely entangled with the old one that it often seems as if the production of the new flagellum occurs exclusively by fission.

6. The extranuclear chromatoid granules of *H. calliphorae* consist of volutin. They are numerous during the preflagellate stage and disappear gradually during the flagellate stage; in the post-flagellate stage they are few in number or altogether absent.

This behaviour suggests that volutin in *H. calliphorae* may act as a sort of nutritive reserve-substance. In degenerating individuals the volutin granules become extremely numerous, an abnormal condition observed also in *Trypanosomata* ("volutinosis"). Consequently volutin seems to play the double part of a nutritive substance, and product of degeneration; this, however, likewise holds for many other nutritive substances (fat, glycogen, etc.).

7. The blepharoplast of *H. calliphorae* shows cyclical changes. During the formation of the post-flagellate stages the achromatic substance is lost and only the chromatic portion remains. The blepharoplast is smallest at this period. The preflagellate stages possess a larger blepharoplast, and this organellum reaches its maximum dimensions during the flagellate stage. I am not able to say whether or not these cyclical changes have any special importance; perhaps they have something to do with the regulation of the normal relation between nucleus and protoplasm.

8. *H. calliphorae* was observed by me only in the gut of the fly and does not seem to be transmitted hereditarily, as is the case in *Crithidia melophagia* (Swingle, 1909; Porter, 1910).

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THE OVA OF *SCHISTOSOMA JAPONICUM* AND THE ABSENCE OF SPINES.

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The observations recently set forth by Dr Leiper[1], and by Dr Sambon in a discussion[1], have led us to re-examine the ova of *S. japonicum* in the material which we have at hand. This material was described previously by one of us[2] together with a report of some comparative measurements of the ova of this species of trematode furnished by Dr Shiga.

We have now re-examined carefully several hundred ova in stained sections of tissues and a great number of others which we have teased free from unstained tissues which have been well preserved in Kaiserling's solution. In no instance did we find the least appearance of a blunt protuberance or spine on the outer envelope of the egg. In one or two instances the embryo itself caused a slight protuberance about the size of the spine represented in Dr Leiper's microphotograph, but without any of the other characteristic points reported by Dr Leiper—for instance the thickening of the envelope about the protuberance.

When examining the ova of *S. mansoni* we have never experienced any difficulty in recognizing the lateral spines, and if the blunt lateral

[1] Leiper, R. T. (III. 1911). Note on the presence of a lateral spine in the eggs of *Schistosomum japonicum*. *Trans. Soc. Trop. Med. and Hyg.* IV. p. 133. (Discussion, p. 135.)

[2] Woolley, P. G. (I. 1906). The occurrence of *Schistosoma japonicum vel cattoi* in the Philippine Islands. *Philippine Journ. Science*, I. p. 83.

spine of *S. japonicum*, as depicted by Dr Leiper, is a distinctive characteristic we should have been able to recognize it in the present series of examinations, even though we had overlooked it in an equally large number of examinations upon which our original measurements were based.

With regard to the similarity of these ova and those of *Agchylostoma duodenale*: the ova of the latter are more nearly ovoid while those of *S. japonicum* are somewhat flattened, so that we would hardly speak of rolling them under the cover glass but rather of turning them over.

THE TRANSMISSION OF *SPIROCHAETA DUTTONI.*

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Although Livingstone, in 1857, suggested that ticks were responsible for the transmission of the Relapsing Fever of Tropical Africa, it was not until 1905 that Dutton and Todd experimentally proved that *Ornithodorus moubata* transmitted this disease. They found that spirochaetes appeared in the blood of monkeys, on which had been fed ticks collected from native huts in the Congo, and as they had previously recognized that spirochaetes were the parasites which caused this fever, the transmission of the disease was thus proved experimentally to be due to *O. moubata.* They also found that the offspring of infected ticks were capable of infecting animals on which they fed, and thereby proved that *O. moubata* is the true intermediate host of *Spirochaeta duttoni*, and that some development must take place in the tick after the spirochaetes have entered the gut. Owing to lack of appliances etc., these two investigators were unable to follow the life-cycle of the parasite within the intermediate host, but they described certain changes taking place in the Malpighian tubules, and the passage of the spirochaetes through the wall of the alimentary canal.

The same year Koch (1905) made some observations on this disease in East Africa, and independently confirmed the work of Dutton and Todd. He found that 5—15 per cent. of the ticks were infected; and also made some observations on the life-history of the spirochaete in the body of the tick. The parasites penetrated the gut-wall into the tissues of the tick, and were found to enter the undeveloped eggs within the ovaries, and Koch figures tangled masses of spirochaetes as occurring in

the egg. He imagined that, after penetrating the gut-wall, some of the spirochaetes made their way to the salivary glands and from there probably were injected into the wound caused by the tick's bite.

The following year Breinl, Kinghorn and Todd (1906) attempted to transmit *S. duttoni* by the bites of *Cimex lectularius*. The bugs were kept at a temperature of about 20° C. and after feeding on infected animals were allowed to feed on healthy monkeys; but though, in all, several hundreds of this insect were experimented with, the results were uniformly negative as regards the transmission of both *S. duttoni* and *recurrentis*.

Nuttall (1907) also attempted to transmit spirochaetes from infected to healthy animals by means of bed-bugs. He found that at 12° C. the spirochaetes lived in the gut of the bug for as long as 120 hours, but when kept at higher temperatures they disappeared much more rapidly. At 25° C. the parasites were all dead within three hours after being ingested into the gut. These experiments, however, were also negative as far as the actual transmission of *S. duttoni* from one animal to another was concerned, although one positive result was obtained with *S. recurrentis* by interrupted feeding on mice.

The same year Fülleborn and Mayer (1907) attempted to transmit this disease by the bite of *Stegomyia fasciata*, but the results were uniformly negative.

In 1907 Möllers gave an account of his detailed experiments on the transmission of *S. duttoni*, in which he showed that infected ticks (*O. moubata*) may feed repeatedly on uninfected animals and yet remain infective. Employing ticks imported from German East Africa, he succeeded in infecting the first ten out of twelve animals on which they were fed. The negative results of the last two experiments were probably due to the fact that very few ticks were left alive and consequently there were not enough to cause any infection. The ticks used in this experiment were fed every two months, so that they remained infective at least eighteen months after their first feed upon an infected animal.

Möllers has also shown that infected ticks fed successively on six "clean" animals, after each feed may lay a batch of infected eggs, the ticks hatched out from which are capable of conveying the infection to the animals they feed upon. Moreover, not only is the infection carried through to the second generation, but also to their offspring; ticks of the third generation being found to be infective, even though their parents had never fed on an infected animal.

Manteufel (1908) showed that *O. moubata* could transmit *S. recurrentis* as well as *S. duttoni*, and Brumpt (1908 *b*) showed that the same species of tick could carry *S. marchouxi* (*gallinarum*). The latter author, however, was unable to transmit the respective spirochaetes of the Algerian and American Relapsing Fevers, by *O. moubata*.

Brumpt (1908*a*) described the existence of a "tick-fever" in Abyssinia which at first was supposed to be transmitted by *O. moubata*, but afterwards Doreau (1908) proved that this was not the case. In a subsequent paper, Brumpt (1908 *b*) proved by experiments on animals, that this spirochaete of Abyssinia was transmitted by *O. savignyi*.

The transmission of *S. duttoni*, *recurrentis* and *novyi*, respectively, by means of *O. moubata*, was successfully accomplished by Neumann (1909), and thus one of the points of distinction between these three kinds of spirochaetes was removed. He also found that *S. duttoni* could occasionally be transmitted from rat to rat by means of the rat-louse *Haematopinus spinulosus*.

The results of all the recorded experiments and observations, both in the laboratories and in nature, have shown, however, that *O. moubata* is probably the only ectoparasite which naturally transmits *S. duttoni*, and also that it is the true intermediate host.

Although the main facts regarding the transmission of this disease are thus well established, the exact manner in which the tick conveys the infection to the animal it feeds upon is in some doubt. The majority of investigators have assumed that the spirochaetes make their way to the salivary glands and from there are directly injected into the wound caused by the tick's bite.

The most important work on this subject, and on the life-cycle of *S. duttoni* within the intermediate host, is that of Leishman (1909, 1910) who brought forward considerable evidence to show that at ordinary temperatures the salivary glands of the tick do not become infected, and that infection probably takes place, either by the regurgitation, or by the excretion, of infective matter from the alimentary canal. In either case the spirochaetes could make their way into the animal through the wound caused by the tick's bite. To test the truth of this hypothesis Leishman performed the following experiment, which may be described in his own words:—

"Nine ticks which I had infected some months before were allowed to bite a monkey. After they had fixed and commenced to feed they were gently removed and placed on another monkey on whom they

were allowed to gorge. The fact of their all having bitten the first monkey was verified by observing the punctures and small ecchymoses which surrounded the bites. As is well known, when these ticks commence to swell they pass a large quantity of clear fluid from the coxal glands (whose anti-coagulating properties I have been able to determine) and some, in addition, pass the white secretion of their Malpighian tubes. These fluids, therefore, bathe the skin of the monkey and almost certainly may penetrate into the subcutaneous tissues through the punctures left when the tick has loosened its hold on its host. The two monkeys, then, although bitten by the same ticks, differed in the following respect: on the first none of this fluid had been passed, while on the other the wounds were bathed in it. Some of the fluid passed on the second monkey was also collected and inoculated hypodermically into a mouse. Careful search of this fluid showed neither spirochaetes nor granules; the latter, however, are so minute that they might readily be missed. The result of this experiment was interesting and suggestive; the first monkey was not infected, while the second monkey and also the mouse developed tick fever, which ran a typical course" (*Lancet*, 1910, p. 13).

In addition a few experiments were performed by isolating the tissues and organs of infected ticks and, after emulsification in saline, injecting the material into white mice. The results were negative in the case of emulsions made from the Malpighian tubes and salivary glands of two ticks known to have been infected and kept at a uniform temperature of 24° C., and also in the case of an injection of the salivary glands of a tick that had been kept at a temperature of 37° C. for two days before the operation. The only positive results were obtained from the emulsions, respectively, of the gut-wall, Malpighian tubules, Malpighian secretion and ovary, of a tick that had been kept at a temperature of 37° C. for two days before dissection.

Leishman's important observations on the morphological changes taking place in the spirochaetes after entering the tick will be considered in a later communication, dealing with the morphology of *S. duttoni.*

Schuberg and Manteufel (1910) have recently discovered that certain individuals of *O. moubata* may acquire an active immunity against infection with *S. duttoni,* and also *S. recurrentis.* About 100 young ticks were infected, half with East African Relapsing Fever and the other half with Russian Relapsing Fever, and afterwards kept at a uniform

temperature of 22° C. About a year later the surviving ticks, of both series, produced no infection when fed upon healthy rats. At certain intervals the ticks were repeatedly fed on uninfected rats but never produced any infection in them. Those which had been infected with *S. duttoni* were then fed upon a rat infected with the same strain and the following month these ticks were again fed upon a healthy animal, but no infection was produced. It was subsequently found that after being taken into the alimentary canal of an immune tick, the spirochaetes were rapidly destroyed and only retained their infectivity (determined by inoculation) for two days. The destruction of the parasites took place in the gut of the tick and the spirochaetes did not penetrate into any of its organs or tissues.

The fact that *O. moubata* may possess an active immunity against the Relapsing Fever of Tropical Africa is of the highest importance from the epidemiological point of view, and also explains the negative results which have followed many of our transmission experiments.

Introduction.

The experiments described in the following pages are a direct continuation of some performed, in 1909, by Nuttall and Fantham, who tried to determine, by means of inoculation experiments, which organs of the tick harboured *S. duttoni.* Owing to a succession of epidemics of another disease amongst the mice used in these experiments, the investigation was never completed, and in 1910, when I commenced to work on this subject, Professor Nuttall kindly placed the records of these unpublished experiments at my disposal. During the past year a number of experiments have been made in order to determine the mode in which the tick transmits spirochaetal infection, and also which organs, or tissues, harbour the parasites.

Although the number of experiments is rather small, yet the results are sufficiently definite to show which organs of the tick are infective, and also to arrive at some conclusions regarding the influence of temperature upon their infectivity. The results which have been obtained support Leishman's view that it is not the salivary glands but the gut which remains infective, and that infection is produced probably as a result of the excretion of infective material from the Malpighian tubules, which enters the open wound caused by the tick's bite.

Technique.

The ticks used in the following experiments came from Uganda and most of them were found to be infected with *S. duttoni.* In all cases, however, the ticks were fed once or twice in the laboratory on mice heavily infected with *S. duttoni,* in order to make certain that they had been exposed to infection. Except in the case of certain experiments described below, the ticks were kept in glass jars at a uniform temperature of about 21° C., and under these conditions remained healthy for several months.

In those experiments undertaken to decide, by inoculation, which organs of *O. moubata* become infected, the tick was carefully dissected in 0·8 % salt solution, and after its organs had been isolated, each was washed in several changes of saline in order to remove any spirochaetes which might have adhered to the outside of the organ. It is very difficult to perform this dissection without rupturing the gut, and therefore this washing had to be very thorough in order to make certain that none of its contents remained on the outside of other organs. After the isolation of the various parts, each organ was emulsified in normal saline solution and injected intraperitoneally into a mouse. Usually films were made from a part of each organ that was injected and, after staining, examined for spirochaetes. The mice used in these experiments were examined daily for about four weeks after the inoculation, and if spirochaetes had not appeared in the blood in this time, it was assumed that no infection had taken place.

The experiments may be divided into two groups according to whether the ticks had remained at a moderate temperature (about 21° C.), or had been maintained at a temperature of 35°–37° C. for some time previous to the dissection.

In the following protocols the results have been arranged as follows:—In column 1, 'Tick material injected,' is given the part of the tick which was inoculated into the mouse. Under 'Result of Inoculation' is expressed by means of a positive (+) or negative (−) sign, whether the mouse became infected or not. Under 'Incubation period' is given the number of days which elapsed, prior to the appearance of spirochaetes in the blood of the mouse. In the last column—'Microscopical examination'—is given the result of the examination of the tick material for spirochaetes, their presence or absence being denoted by a +, or − sign, respectively.

Infectivity of the organs of ticks kept at moderate temperatures.

Exp. 1. (Nuttall and Fantham) 16. II. 09. *O. moubata*, immature ♀, last fed 12. XI. 08. Kept at a uniform temperature of 20° C., and finally, for 24 hours at 24° C.

Tick material injected	Result of inoculation	Incubation period (days)
Coxal fluid + excrement	+	8
Malpighian tubules	+	8
Salivary glands	–	–
Ovary	+	21

Exp. 2. (N. & F.) 23. III. 09. *O. moubata*, adult ♀, last fed 12. XI. 08. Kept at a uniform temperature of 20° C.

Tick material injected	Result of inoculation	Incubation period (days)
Gut + contents	+	6
Malpighian tubules	+	8
Salivary glands	+	6
Ovary	+	15

Exp. 3. *O. moubata*, immature ♀, fed on infected mouse 8 days previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	6	+
Malpighian tubules	–	–	–
Salivary glands	–	–	–
Ovary	–	–	–

Exp. 4. *O. moubata*, adult ♂, fed on infected mouse 21 days previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	11	–
Malpighian tubules	–	–	–
Salivary glands	–	–	–
Testis	–	–	–

Exp. 5. *O. moubata*, immature ♀, fed on infected mouse 7 weeks previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	–	–	–
Malpighian tubules	Mouse died, negative	–	–
Salivary glands	–	–	–
Ovary	–	–	–

Exp. 6. *O. moubata*, immature ♀, fed on infected mouse 5 months previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	7	–
Malpighian tubules	+	14	–
Salivary glands	–	–	–
Ovary	+	7	–

Exp. 7. *O. moubata*, adult ♂, fed on infected mouse 7 days previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	–	–	–
Malpighian tubules	–	–	–
Salivary glands	–	–	–
Testis	–	–	–

Exp. 8. *O. moubata*, immature ♂, fed on infected mouse 7 months previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	9	–
Malpighian tubules	+	7	–
Salivary glands	+	7	–
Testis	+	7	–

Exp. 9. *O. moubata*, immature ♀, fed on infected mouse 4 months previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	–	–	–
Malpighian tubules	–	–	–
Salivary glands	–	–	–
Ovary	–	–	–

Exp. 10. *O. moubata*, adult ♂, fed on infected mouse 4 months previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	9	–
Malpighian tubules	–	–	–
Salivary glands	–	–	–
Testis	+	7	–

Exp. 11. *O. moubata*, adult ♀, fed on infected mouse 4½ months previously. Kept at a uniform temperature of 21° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	8	–
Malpighian tubules	+	8	–
Salivary glands	–	–	–
Ovary	+	7	–

On summarizing the results described above, it will be seen that out of the eleven experiments, three were entirely negative, *i.e.*, the ticks were immune to spirochaetal infection.

The results of the eight positive experiments have been arranged in the form of a table, in which is given the total numbers of positive

and negative results, obtained respectively from the various parts of the ticks.

Tick material injected					Result of inoculation	
Coxal fluid + excrement (Exp. 1) / Gut + contents			...	...	8+	0–
Malpighian tubules	...	...	...	...	5+	3–
Salivary glands	...	...	...	...	2+	6–
Sexual organs	...	...	...	...	6+	2–

It is important to remember that in these experiments a negative result is much less liable to error than a positive one. Whilst isolating the various organs, it is almost impossible to prevent the contents of the gut from coming in contact with the other parts of the tick, and although the latter were always carefully washed to try to remove any such contamination, it is difficult to make certain of the removal of all traces of infective material from the outside of the organs. The two positive results which have been obtained with the salivary glands, in experiments 2 and 8, can be explained on the supposition that these organs were contaminated with the gut + contents. In experiment 2 the incubation period (6 days) of the mouse injected with the contents of the gut is exactly the same as that injected with the salivary glands; and in experiment 8 there is only two days difference between the corresponding periods.

Infectivity of the organs of ticks kept for some time previous to dissection at a high temperature (35—37° C.).

Exp. 12. (Nuttall and Fantham) 24. II. 09. *O. moubata*, small adult ♂, last fed 12. XI. 08. Kept at a uniform temperature of 21° C.; finally, for 20 hours at 37° C.

Tick material injected	Result of inoculation	Incubation period (days)
Gut + contents	+	10
Malpighian tubules	+	12
Salivary glands	+	10
Testis	+	10

Exp. 13. (N. & F.) 25. II. 09. *O. moubata*, small adult ♂, last fed 12. XI. 08. Kept at a uniform temperature of 21° C.; finally, for 44 hours at 37° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	6	+
Malpighian tubules	+	6	+
Salivary glands	+	6	–
Testis	+	5	+

Exp. 14. (N. & F.) 15. III. 09. *O. moubata*, adult ♀, last fed 12. XI. 08. Kept at a uniform temperature of 21° C.; finally, for 42 hours at 37° C.

Tick material injected	Result of inoculation	Incubation period (days)
Gut + contents	+	8
Malpighian tubules	+	7
Salivary glands	Mouse died 16. III. 09	–
Ovary	+	8

Exp. 15. *O. moubata*, adult ♂, fed on infected mouse 5 weeks previously. Kept at a uniform temperature of 21° C.; finally, for 40 hours at 37° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	5	+
Malpighian tubules	–	–	–
Salivary glands	+	5	–
Testis	+	7	+

Exp. 16. *O. moubata*, immature ♂, fed on infected mouse 6 weeks previously. Kept at a uniform temperature of 21° C.; finally, for 48 hours at 37° C.

Tick material injected	Result of inoculatiou	Incubation period (days)	Microscopical examination
Gut + contents	–	–	–
Malpighian tubules	Mouse died, negative	–	–
Salivary glands	–	–	–
Testis	–	–	-

Exp. 17. *O. moubata*, adult ♂, fed on infected mouse 7 weeks previously. Kept at a uniform temperature of 21° C.; finally, for 3 days at 36° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	–	–	–
Malpighian tubules	–	–	–
Salivary glands	Mouse died, negative	–	–
Testis	–	–	–

Exp. 18. *O. moubata*, immature ♂, fed on infected mouse 5 months previously. Kept at a uniform temperature of 21° C.; finally, for 3 days at 36° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	5	+
Malpighian tubules	+	5	+
Salivary glands	+	7	?
Testis	+	7	+

Exp. 19. *O. moubata*, adult ♀, fed on infected mouse 7 months previously. Kept at a uniform temperature of 21° C.; finally, for 3 days at 36° C.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	6	+
Malpighian tubules	+	7	+
Salivary glands	+	7	+
Ovary	+	5	+

In this series of experiments also it will be noticed that two of the ticks were uninfected even though they had been fed some time previously on a heavily infected animal.

The results of the remaining six positive experiments have been summarized in the following table:—

Tick material injected				Result of inoculation	
Gut + contents	...	...	...	6+	0–
Malpighian tubules	...	...	...	5+	1–
Salivary glands	...	...	...	5+	1?
Sexual organs	...	...	...	6+	0–

In the above described experiments five out of the nineteen ticks used were uninfected, and therefore must be considered to have been immune to infection with *S. duttoni*. These results confirm Schuberg and Manteufel's (1910) observations on the immunity of certain individuals of *O. moubata* against spirochaetal infection; and also show that a rather large proportion (about 30 %) of the ticks possess this immunity. When performing transmission experiments, or attempting to follow the life-history of the spirochaetes in the intermediate host, it is important to remember this fact.

Discussion of the positive experiments.

(*a*) The 'gut + contents' were found to be infective in all cases, and in many instances the spirochaetes could be seen by means of microscopical examination. There is no difference between the ticks which had been kept at 21° C. and those which had been kept for some time at 37° C., as far as the infectivity of the 'gut + contents' is concerned, except that the incubation period in the latter is, on the whole, one or two days shorter. There is a marked difference in the result of the microscopical examinations, however, for whereas, in the case of ticks kept at 21° C., spirochaetes were only seen in one case, when the tick had fed on an infected mouse eight days previously, parasites were invariably found in the 'gut + contents' of those which had been kept for some time at about 36° C.

These experiments clearly prove that the gut together with its contents always harbours a spirochaetal infection, except in the case of those ticks which are immune.

(*b*) The Malpighian tubules are less constantly infected than the 'gut + contents,' but nevertheless there are five positive results against

three negative ones in the case of ticks which had been kept at 21° C. It may reasonably be assumed, therefore, that at moderate temperatures these organs often harbour the infection, although spirochaetes were never found by microscopical examination. On the other hand, in the case of those ticks which had been kept at 36° C., the Malpighian tubules, with one exception, were all infective and spirochaetes were found in three out of the four examined microscopically. The result of incubation in this case, therefore, is to increase the possibility of the Malpighian tubules harbouring infection and also to cause the appearance of spirochaetes in their tissue. Moreover, as in the case of the 'gut + contents,' the incubation period is slightly curtailed.

(*c*) The salivary glands are probably never infected in ticks which have been kept at moderate temperatures. The two positive results which have been obtained out of eight experiments may be explained on the supposition that the salivary glands had been contaminated by infective material from the gut. On the other hand, the effect of raising the temperature of the tick is markedly shown in this case, for, with one doubtful exception (the mouse died before it had time to become infected), the salivary glands were always infective.

The results of the microscopical examination for spirochaetes are not definite. In the case of ticks which had been kept at 21° C. the parasites were never found, but even in the case of the incubated ticks, whose salivary glands were infective, in only one case were spirochaetes found. It is possible that they are present only in very small numbers and might easily be overlooked, but the possibility of their being present in another form is also to be considered.

(*d*) The sexual organs of ticks which had been kept at 21° C. are generally infective, for out of eight experiments only two were negative. When the ticks are incubated at 36° C. the sexual organs were always found to be infective and therefore there is a slight increase in their infectivity as a result of raising the temperature. The incubation period is also slightly curtailed.

The microscopical examination of the sexual organs for spirochaetes was uniformly negative in the case of ticks kept at 21° C., but when the latter had been incubated for some time at about 36° C., spirochaetes were invariably found in considerable numbers.

The effect of raising the temperature in this case, therefore, is to increase the infectivity of the sexual organ, and also to cause the development of spirochaetes in its tissues.

Infectivity of eggs.

The infectivity of a certain proportion of the eggs laid by an infected parent is proved by the fact that a varying percentage of the nymphs, which hatch out from them, are capable of producing infection when they feed on a healthy animal. Three experiments, however, were performed by injecting the contents of eggs into mice.

In each case the parent and the eggs had been kept at a uniform temperature of 21° C. and in none of them were spirochaetes detected by microscopical examination[1].

Exp. 1. Mouse, injected with the contents of 12 eggs, laid by an *O. moubata* fed on an infected mouse 20 days previously, and kept at a uniform temperature of 21° C. Result—negative.

Exp. 2. Mouse, injected with the contents of 12 eggs, laid by an *O. moubata* fed on infected mouse five weeks previously, and kept at a uniform temperature of 21° C. Result—positive, the mouse becoming infected after four days incubation period.

Exp. 3. Mouse, injected with the contents of 10 eggs, laid by an *O. moubata* fed on infected mouse five months previously, and kept at a uniform temperature of 21° C. Result—positive, the mouse being infected after seven days incubation period.

These results are sufficient to show that the infected eggs are capable of producing an infection when injected into mice, even though they have been kept at a moderate temperature (21° C.) and do not contain recognizable spirochaetes.

Coxal fluid.

In three cases the coxal fluid was carefully collected from infected ticks, whilst they were feeding, and injected into mice. The results were negative in each case, and therefore it is probable that the coxal fluid does not contain any infective material. It plays an important part, however, in the production of infection when the tick feeds, as by means of this fluid, which bathes the under surface of the tick, the infective material contained in the excrement is carried into the wound caused by the tick's bite.

[1] In every case we only give the results of microscopical examination for *typical* spirochaetes. Other forms of the parasite will be considered later.

THE METHOD IN WHICH AN INFECTED TICK PRODUCES SPIROCHAETAL INFECTION IN THE ANIMAL IT FEEDS UPON.

The results of the inoculation experiments with ticks that had been kept at 21° C., show that the salivary gland probably never becomes infected at this temperature, and yet these ticks produced spirochaetal infections, when they were fed upon healthy mice, as readily as those ticks that had been kept for some time at 36° C., and whose salivary glands were infected.

It is evident, therefore, that the salivary glands are not usually responsible for any spirochaetal infection that may follow the bite of a tick infected with *S. duttoni.*

In order to decide definitely the exact mode of infection, the following experiment was performed and the results are given in detail.

Three adult specimens of *O. moubata,* which had been kept at a uniform temperature of 21° C., were allowed to feed on a healthy mouse. After feeding, each of the ticks passed one or two drops of the clear coxal secretion and also voided some of their white excrement (Malpighian secretion) from the anus. The mouse became infected after an incubation period of 10 days.

Some of the coxal secretion from these ticks was injected into a mouse but no infection was produced. On the other hand, a mouse which was injected with some of the white excrement voided by the ticks, became infected after an incubation period of 11 days, which is only one day longer than that of the mouse used for the feeding experiment (10 days).

Each of the three ticks used in this experiment was dissected and its organs injected into mice, in the manner described above (p. 138).

Tick 1. Adult ♀. Was immune to *Spirochaeta duttoni*, as none of the mice injected from it became infected.

Tick 2. Adult ♂.

Tick material injected	Result of inoculation	Incubation period (days)	Microscopical examination
Gut + contents	+	9	–
Malpighian tubules	–	–	–
Salivary glands	–	–	–
Testis	+	7	–

Tick 3. Adult ♀.

Gut + contents	+	8	–
Malpighian tubules	+	8	–
Salivary glands	–	–	–
Ovary	+	7	–

These results prove conclusively that in this case the salivary glands were not responsible for the infection that followed this feeding experiment, as they were all uninfected. Moreover, the injection of the coxal secretion was not followed by any infection; therefore it was evidently not the infective agency.

The only possible mode of infection in this case was either the regurgitation of infective material from the gut, or the excretion of the white Malpighian secretion, and the latter was proved by inoculation to be infected. This white secretion is voided by the great majority of ticks, usually when they are finishing feeding. It is voided by the first stage nymphs as often as in the more advanced stages, and is invariably infective when it comes from infected ticks. When the coxal fluid is secreted it usually runs underneath the body of the tick and mixes with the Malpighian secretion, the latter thereby becoming diffused and brought into the open wound caused by the tick's bite. There is no doubt that under such conditions a susceptible animal would become infected, and the only doubtful point is whether this is the invariable method by which animals become infected with spirochaetes, after the bite of infected *O. moubata.* From a consideration of the inoculation experiments described in the first part of this paper, it seems probable that it is the only mode of infection for, at ordinary temperatures, the salivary glands do not become infected, whereas the gut and its contents are invariably infective, except in the case of immune ticks.

In addition we have repeated with success Leishman's experiment (in our case using mice) of allowing ticks to feed on an animal for a short period and then transferring them to another in order to complete their meal. Under such conditions the ticks have not time to void any of the Malpighian secretion on the first animal and it never becomes infected; on the contrary, when the same ticks are allowed to feed to repletion on a second animal it usually becomes infected, in this case the ticks having voided their excrement and also some of the coxal fluid.

In conclusion, therefore, we should like to repeat that our results entirely confirm Leishman's suggestion that, in the case of *O. moubata* infected with *S. duttoni*, the infection they may produce in man and other animals is the result of the excretion of infective material from the Malpighian tubules and gut, which enters the open wound caused by the tick's bite.

I am indebted to Prof. Nuttall for suggesting to me the line of investigation to which this paper relates and for placing his material and results at my disposal.

Summary of Results.

(1) About 30 % of the *O. moubata* from Uganda have been found to be immune to infection with *S. duttoni.*

(2) When infected ticks are kept at ordinary temperatures (about 21° C.), the following parts of the body are found to harbour the infection :—gut + contents, sexual organs, Malpighian tubules, excrement.

The following parts were found to be uninfective :—salivary glands, coxal fluid.

(3) When infected ticks are kept at a temperature of about 35° C. for two or three days previous to dissection, all the organs of the body, including the salivary glands, are found to be infective. Moreover the incubation periods that elapse before the appearance of spirochaetes in the injected mice are shorter than in the case of the corresponding experiments with ticks that had not been kept at a high temperature.

(4) No spirochaetes have been detected in any of the organs of infected ticks that had been kept at a temperature of about 21° C. When a tick ingests blood containing spirochaetes, the latter persist in the lumen of the gut for periods varying from a few days up to as long as four weeks; usually, however, they disappear from the gut in the course of nine or ten days.

(5) When infected ticks are heated to a temperature of 35° C. for two or three days, spirochaetes reappear in the lumen of the gut, and also appear in all the organs of the tick, and in the coelomic fluid.

(6) The spirochaetal infection that may follow the bite of an infected *O. moubata* results from the entrance of the infective material, excreted by the tick whilst feeding, into the open wound caused by the tick's bite. It is not the result of the inoculation of infective material from the salivary glands.

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A REPLY TO MISS PORTER'S NOTE ENTITLED "SOME REMARKS ON THE GENERA *CRITHIDIA*, *HERPETOMONAS* AND *TRYPANOSOMA*."

By H. M. WOODCOCK, D.Sc.,
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In a recent number of *Parasitology*[1], Miss Porter, under the above heading, complains because, in the *Zoological Record* for 1909 (*Protozoa* division, p. 60), I put the generic names *Crithidia* and *Herpetomonas* in inverted commas, when applied to certain species; on this ground she charges me with allowing my personal opinions to influence me in my compilation of the *Record*, my opinions being, according to her, "extreme and decidedly opposed to the opinions of those who have worked personally on the parasites in question." Incidentally, Miss Porter makes it appear that my opinions *are* extreme and of little or no value.

I should not have troubled to reply to Miss Porter's remarks if she had not criticised my conduct of the Protozoa division of the *Record*. But as this criticism is calculated to mislead any general Readers who may be interested in the *Zoological Record* but are not particularly conversant with the point at issue, I propose to justify myself to them. I am sorry that in doing so I shall have to show that Miss Porter's method of criticism is not straightforward, but, on the contrary, distinctly oblique.

To begin with, I will refute the charge that my opinions are extreme. In the first place, Miss Porter quotes *part* only of the references to *Crithidia* and *Herpetomonas* given on p. 60 of vol. 46 of the *Record*. Immediately before the species she quotes, under the two respective headings, are the following references: "*Crithidia gerridis*, general account, Porter," etc.; and "*H.* (i.e. *Herpetomonas*) *vespae* sp.n., *H. jaculum*, Porter," etc. That is to say, the generic names are *not* put in

[1] Vol. IV. pt. 1, 1911, p. 22.

inverted commas when the references are to accounts of such parasites from non-blood-sucking Insects—including, it will be seen, the reference to Miss Porter's own paper. In these cases they are given as indicating real genera. It is only where such forms are described from blood-sucking hosts that the inverted commas are used. Again, to leave the *Record*, in my own article on Avian Haemoprotozoa, to which Miss Porter also refers, on p. 713 is the following sentence: "One or two cases have been described, however, of the occurrence of crithidial forms in what are alleged to be non-sanguivorous Insects (*e.g. C. gerridis*, from *Gerris fossarum*, Patton); such parasites may apparently be regarded as true Crithidiae, by which we may understand Flagellates that have developed a trypanomonad condition but which are restricted to an Invertebrate host." This sentence is on the page on which I mention my discovery of a Trypanosome in the blood of the sheep. Further, I quote the following sentences (p. 244) from my article, written about the beginning of 1908, on the Haemoflagellates in Lankester's *Treatise on Zoology*, vol. I. Protozoa, to which Miss Porter also makes reference: "Hence, summing up, there can be little doubt that certain of these parasites of mosquitoes, especially those with trypaniform characters, are connected with some Vertebrate host, just as are those of other blood-sucking Invertebrates. At the same time, it is also probable that some of the (typical) Herpetomonads found (*e.g.* those occurring in larvae, such as Patton's form, also certain forms described by the Sergents) are simply and primarily parasites of the Insect. Lastly, it is, of course, possible that such a parasite may have developed a trypaniform condition as an adaptation to the food of a sanguivorous Insect, without, however, having become able to live in the Vertebrate host; but so far no example of such a case is definitely known." (I may say in passing, with regard to the last sentence, that I would not go so far in that direction to-day; as I discussed in my recent paper on Avian parasites, I consider the occurrence of a trypaniform phase in a blood-sucking Insect is almost conclusive indication that the parasite in question is a Trypanosome.)

It is extremely doubtful, indeed, whether even any of the trypanomonad ("crithidial") forms found in blood-sucking Insects can be regarded as independent. It is true that I have not worked personally on genuine crithidial or herpetomonad parasites from non-blood-sucking Insects. But I have paid considerable attention to the characteristic developmental phases of blood-Trypanosomes, which occur both in cultures and in the Invertebrate hosts. And knowing what I do about such forms, as soon as I observed and studied "*Crithidia*" *melophagia* occurring

in several sheep-keds taken from the same sheep, the conclusion was irresistibly suggested to my mind that this parasite could be nothing else than the trypanomonad form of a Trypanosome in the sheep. At Miss Robertson's suggestion, she and I instituted a searching examination of the blood of that particular sheep, with the result that I found the Trypanosome whose presence was suspected.

Having regard to all the known facts, the only reasonable and logical view to take is that these parasites in blood-sucking Insects are phases of some Trypanosome, *especially where the Insects feed on animals known to harbour a Trypanosome* (*e.g.* rat-fleas, mole-fleas, sheep-keds, etc.). This is the view of commonsense. So far as proof goes, this view has already been conclusively proved by the work of Minchin and Thomson, in the case of the parasites of the rat-flea; and sooner or later it will be proved in other cases. Most assuredly, however, the *onus probandi* lies rather on those who hold the opposite view; and, so far as I am aware, neither Miss Porter nor anyone else has attempted to prove that any of these "*Crithidiae*" in blood-sucking Insects are true independent forms.

Secondly, the above view represents not only *my* personal opinion. It is also the opinion of the great majority of Protozoological workers, of whom I need only refer to Prof. Minchin, whose name is generally recognized as that of one of the foremost authorities on Trypanosomes and allied parasites. Miss Porter mentions Swingle as being opposed to this view. Swingle is certainly not now opposed to it; he is at any rate quite open-minded, and indeed if anything *in favour of* my view. In a recent paper on the transmission of *T. lewisi* by rat-fleas[1], etc., he says on p. 131, with reference to Strickland's account of *Crithidia ctenophthalmi*: "It should be noted that these fleas all came from rats infected with Trypanosomes, which fact strongly suggests that his Crithidiae were really transformed Trypanosomes." Again, in a footnote to p. 141, with reference to my discovery of the Trypanosome in the sheep, he says: "While there is considerable evidence favouring his conclusion, it seems to me there is still a possibility that *C. melophagia* is a true Insect Flagellate, which has never been successfully introduced into the sheep's blood." "Considerable evidence" in favour, it will be noted, and only a "possibility" against. Lastly, I am sure Dr Swingle will not object to my quoting a letter which I received lately from him: "I received your excellent article on Avian Haemoprotozoa soon after I had sent my work to the printer. It is interesting

[1] *Journal Inf. Diseases*, VIII. 1911, p. 125.

that you found a Trypanosome in the blood of the sheep. Since the Trypanosomes change over into Crithidiae in the digestive tract of Insects, it would not be surprising if experiment should reveal the fact that *C. melophagia* is got from the blood of the sheep."

From what I have shown above, therefore, two things will be clear to those who are really interested in the *Zoological Record*. In the first place, there is no truth in the assertion that my opinions on the subject are extreme; on the contrary, they agree with the view which most workers on it hold. Secondly, I am correct in having used and in continuing to use the terms *Crithidia* and *Herpetomonas* in inverted commas, when applied to parasites from blood-sucking Insects, signifying thereby that, according to our present knowledge, such forms are in all probability *not* to be regarded as belonging to truly independent genera, but are merely developmental phases of some Trypanosome. At any rate, I have received the assurance of the Secretary of the Zoological Society and of others on the matter. A Recorder is allowed the use of his own judgment in the case of debated points; and I can truthfully say that I endeavour to keep mine unbiassed and to compile the Protozoa division of the *Record* in accordance with the general tendency of opinion.

In conclusion I have only to say that the above justification is not intended as a personal reply to Miss Porter. As between Protozoologists, the only comment I should make upon her note would be in terms very similar to those in which I referred to Capt. Patton's "criticism" of my article on the Haemoflagellates, in my paper on Avian Haemoprotozoa, p. 715.

FURTHER REMARKS ON THE GENERA *CRITHIDIA*, *HERPETOMONAS* AND *TRYPANOSOMA*, AND DR WOODCOCK'S VIEWS THEREON.

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Through the courtesy of the Editors of *Parasitology* I have been favoured with an advance proof of the remarks of Dr Woodcock on a former note of mine relating to the procedure of the Recorder of the Protozoa section of the *International Catalogue of Scientific Literature* and the *Zoological Record*. In my article I raised three points. (1) In certain cases where the generic names *Crithidia* and *Herpetomonas* were placed in inverted commas in the Catalogue, I stated that *no* direct evidence existed to justify such a procedure. (2) I protested against such meddlesomeness on the part of the Recorder. (3) I stated that the Recorder, who had not worked on the parasites themselves, held, consequently, extreme views. Dr Woodcock's so-called reply has afforded me considerable amusement and it is a matter of surprise to me that he has been so ill-advised as to write such a poor rejoinder—he must know that he has made no answer to my remarks. That the weakness of his position is well known to him is shown by his recourse to the tactics of vituperation and innuendo, where he remarks that my method of criticism is "not straightforward but, on the contrary, distinctly oblique." Dr Woodcock has no case and so has adopted metaphorically the old, old method of "abusing the plaintiff's attorney," in the shape of discussing my own papers on parasites not originally under discussion. I am sorry that one who apparently claims to be an authority is reduced to such second-rate methods. While I regret the necessarily severe character of this, my rejoinder, it is impossible to deal otherwise with the numerous side issues raised by Dr Woodcock.

To go to the heart of the matter: I ask again, What special evidence is there that *Crithidia hystrichopsyllae* is a trypanomonad

phase of a trypanosome? What special evidence is there that *Herpetomonas ctenophthalmi* is a phase of a trypanosome? Similarly what evidence is there regarding *C. melophagia, C. nycteribiae, C. simuliae,* and *C. tabani* respectively being stages of trypanosomes? Who has found the respective trypanosomes and where are they described? Who has witnessed the metamorphosis of either of the organisms in each direction from *Crithidia* to *Trypanosoma* and from *Trypanosoma* to *Crithidia,* and experimentally proved the identity of the forms? Dr Woodcock in his solicitude for the general reader would have been well advised to answer these questions. Instead, in all courtesy, I would state that he has raised many side issues—and certain personalities which I return to him. Regarding *C. tabani* he might read a recent paper (April, 1911) dealing with the *Crithidia* of Uganda Tabanids by Sir D. Bruce and colleagues which paper does not support his (Woodcock's) views.

Dr Woodcock blames me for quoting only part of his references to *Crithidia* and *Herpetomonas.* Of course I challenged his use of inverted commas in certain specified cases, his method of dealing with other species about which no sane person would quibble was no concern of mine. But Dr Woodcock when discussing *Herpetomonas jaculum* in *Nepa cinerea* lays himself open to criticism, either of sheer ignorance of the feeding habits of *Nepa*—a blood-sucker attacking any blood-containing animal to which it can attach itself; or to lack of constancy in his argument as to his method of treatment; or to carelessness; for he states that it is "only where such forms are described from blood-sucking hosts that the inverted commas are used." Again, the conduct of Dr Woodcock in so insulting the general intelligence of his readers by raising side issues regarding *C. gerridis* in a non-sanguivorous insect is reprehensible. Incidentally he says *Gerris* is "alleged" to be non-sanguivorous. Why this gratuitous doubt should be set forth is incomprehensible when thoroughly capable observers have recorded their observations on the habits of the bug, a most important point when dealing with the life-history of its parasites. Had Dr Woodcock placed the name *C. gerridis* within inverted commas, he might, by now, have been restricted in his movements as quite beyond the pale of commonsense. His trick of raising side issues and setting them up as "men of straw" merely to "be knocked down again," while he hopes for the plaudits of his readers, will not deceive scientific investigators. Dr Woodcock has made the mistake of belittling the intelligence of his readers and co-workers. Let him tell us what direct evidence there is for suggesting that, for example, *C. simuliae* is a stage in the life cycle

of a trypanosome, and until he can tell us this, let him attend to his own affairs and not meddle with other investigators' work.

Personally, I fail to see what the lengthy quotations from Woodcock's paper on Avian Haemoprotozoa, based on hypothesis and analogy, have to do with the points originally raised by me. I am sorry to see Dr Woodcock descend to the borderland of personalities. What his opinions are of my own work is a matter of indifference to me. His mention of the names of Professor Minchin, Miss Robertson, Capt. Patton and Dr Swingle may please him, but it has no relation to the argument. I do not intend to discuss in full his remarks relating to these workers; they have not yet published anything that supports him in answering the specific questions I raised. Regarding Professor Minchin's opinions, however, I would remind Dr Woodcock that his esteemed chief has not yet published anything in support of his view—it is not to be expected that the Professor will support fully in print any such crude hypotheses. Regarding *C. melophagia*, however, I may say that the Professor in his public lectures only last year, gave a synopsis of my work on this parasite, and needless to say there was no mention of such nonsense as an "onus probandi" to show it was not a developing vertebrate trypanosome.

Dr Woodcock makes a sweeping (and illicit) generalisation in stating that these parasites in blood-sucking Insects are "phases of some Trypanosome." He then states that this view, which according to him is the "only reasonable, logical" and "commonsense" one, "has been conclusively proved by the work of Minchin and Thomson, in the case of the parasites of the rat flea." Proof! Conclusively proved! I beg to differ and differ emphatically. Does Dr Woodcock know the meaning of the word "prove"? I think not. The life cycle of *Trypanosoma lewisi* has been described in the rat flea, *Ceratophyllus fasciatus*, and the description of the cycle applies to *T. lewisi* only and to no other parasite. Further, different life cycles of *T. lewisi* in the same flea have been given by Minchin and Thomson on the one hand, and by Swellengrebel and Strickland on the other. How far is Dr Woodcock—or any one else—at liberty to generalise on a single instance? Of course, Dr Woodcock never stops to consider the great danger of rearing general hypotheses on a few instances that are really "random samples." I commend the scientific aspect of "random sampling" to his careful study. The descriptions, then, of the life cycle of *T. lewisi* do not prove anything in general. So much for Dr Woodcock's "reasonable and logical view" and "view of commonsense." Dr Woodcock should know that *C. tabani*, *C. melophagia*, etc.

having been described as and named *Crithidiae* must be considered as such until it can be shown experimentally that they are otherwise. So much for Dr Woodcock's "onus probandi."

While careful study seems to indicate that the simplest form of the Trypanosomatidae is *Herpetomonas*, then increase of complexity leads to *Crithidia* and still further evolution to *Trypanosoma*, yet this is only a probable line of evolution, suggestive it is true, but not proven. Analogy is often misleading. Amphibia such as frogs and newts pass through a fish stage when they are tadpoles; but fishes on that account are not to be regarded as merely stages in the life cycle of Amphibia: the same holds true of *Crithidia* and *Trypanosoma*. The value of analogy in dealing with the development of Trypanosomes has been ably summarised by Sir David Bruce and his co-workers in their recent paper (May, 1911) dealing with the cycle of *T. gambiense* in the tse-tse fly, *Glossina palpalis* (page 517). These workers find that *T. gambiense* in *G. palpalis* practically always retains the trypanosome facies, and that "Crithidia-like forms are exceedingly rare." Further, they state that "because one kind of development takes place with one species of trypanosome and one species of tse-tse fly, it by no means follows that the same thing will occur either with another species of trypanosome or another species of fly. Each combination must be worked out separately and nothing left to analogy."

Dr Woodcock has stated that I have not attempted to prove that any of these *Crithidia* in blood-sucking Insects are true independent forms. I beg to differ. The statement is inaccurate and merely reveals the loose way in which current literature is read. In my paper on *C. melophagia*, I regard the parasite as independent, that is, it has nothing to do with a vertebrate Trypanosome, for it encysts, and other keds by ingesting the cysts become infected. This has been shown experimentally on keds removed from the sheep. Further, the action of vertebrate blood, whether it be that of the sheep or of man, merely stimulates the *Crithidia* to divide (a most important observation which I commend to Dr Woodcock's notice), no "trypaniform" stages were ever found in the keds, though hundreds were dissected during a prolonged period. The life cycle of *C. melophagia* is complete in itself, with pre-flagellate, flagellate and post-flagellate stages (terms which I notice Dr Woodcock never uses—apparently preferring such fantastic and unnecessary ones as "trypanomonad[1]") and there is a true hereditary

[1] Apparently this term is intended to have the same significance as "crithidial," a well-established term. Why, then, should Woodcock coin another term?

infection. *Herpetomonas jaculum,* about whose host Dr Woodcock apparently has such incorrect views, is also stimulated by vertebrate blood to divide.

The finding of a trypanosome in the blood of a sheep makes no difference—at present—to the matter. If Dr Woodcock is so sure that the trypanosome he discovered is connected with *C. melophagia* in the sheep ked, let him *prove* it, not rush into print with "possibilities," which he further uses to edit the names of parasites described by other investigators, in an International Catalogue. *C. melophagia,* considered as an independent organism long before he knew anything about it, must now, according to our worthy, be shown not to be a developing Trypanosome. I commend him for his logic.

In connection with the subject of flagellates of blood-sucking insects, perhaps I may be permitted to draw attention to a research of mine (about to be published) on *Crithidia pulicis,* n. sp. from the human flea, *Pulex irritans. Crithidia pulicis* has the typical life-history, pre-flagellate, flagellate and post-flagellate stages are produced, and the method of infection is contaminative. The fleas were bred for three generations before being dissected, the third generation being used, and the breeding was carried out in special "flea proof" cages of my own design upon my own body. There has been no evidence of hereditary infection. There is no doubt whatever that I am not a victim of trypanosomiasis, and this has been attested by many highly competent workers on human trypanosomiasis. Human fleas, obtained from different parts of England, and their progeny up to the third generation have been examined and *C. pulicis* found in them. According to Dr Woodcock's "generalisation," an enormous number of people in the British Isles—to say nothing of Continental nations—must be the unwilling hosts of some hitherto unknown trypanosome. Yet though thousands of blood smears have been made and examined annually in hospitals and scientific institutes and numerous blood cultures are prepared for various medical purposes, the occurrence of the vertebrate trypanosome yet remains unrecorded, though the unwilling victims of flea bite may number millions.

However, Dr Woodcock, holding the views he does, will doubtless now search for some hitherto undiscovered human trypanosomes, spread by blood-suckers such as sheep keds, water boatmen, water scorpions, etc., all of which feed greedily on human blood when they get the chance. I hope he will take up these researches. Their prosecution at any rate will preserve the protozoological world from a few of his wild hypotheses for the time being. We have had a surfeit of crude hypotheses and

coining of new superfluous terms lately, and can well do without "trypanomonad," "trypaniform," "onus probandi," etc., for a time.

Regarding the quotation from Swingle's paper (p. 131) I would point out that *Crithidia ctenophthalmi* was described from *Ctenophthalmus agyrtes* while the life history of *T. lewisi* has been followed in *Ceratophyllus fasciatus*. It does not then necessarily follow nor indeed "strongly suggest" that *C. ctenophthalmi* is a stage in the life history of *T. lewisi*. Even supposing it is shown later that *T. lewisi* may wholly or partially develop in *Ctenophthalmus agyrtes*, it is quite possible that some of the fleas may have a mixed infection of *C. ctenophthalmi* and developing forms of *T. lewisi*. I have reason to believe that *C. ctenophthalmi* and crithidial forms of *T. lewisi* can be distinguished morphologically, but as I am not describing these parasites—that work belonging to another—I am not at liberty to say more, and I have no wish to emulate Dr Woodcock's questionable example of being keen to edit other people's work.

Dr Woodcock gives a quotation from p. 141 of Swingle's paper. The quotation is from a footnote and is incomplete. The sentence regarding *C. melophagia* printed in Swingle's paper runs as follows:— "While there is considerable evidence favouring his [Woodcock's] conclusion, it seems to me that there is still a possibility that *C. melophagia* is a true insect flagellate which has never been successfully introduced into the sheep's blood, *and that the trypanosome which he* [*Woodcock*] *discovered is an entirely distinct form.*" I have italicised the part of Swingle's statement that Woodcock has omitted, and leave my readers to draw their own conclusions as to Swingle's opinion, and as to the honesty of the gentleman who has accused me of "oblique" methods. Further, I am glad that I am not yet reduced to making unauthorised quotations from private letters in support of my case, but after consideration of the above method of partial quotation, it is possible that if the full context of Swingle's letter were given, we should again find something of the same nature. Incidentally, I, too, count Dr Swingle among my friends and have received letters from him relating to this subject as recently as May 26th, but I prefer to regard private letters as such and not to use them either *in toto* or in mutilated form to support any public controversy or to "puff" my own work.

Dr Woodcock refers to his knowledge of the cultural forms of trypanosomes. I beg to differ as to the value of interpretations placed on organisms under quite unnatural conditions. It seems that Dr Woodcock has changed his views on the value of cultural forms of

trypanosomes, as well as on other protozoological matters. A few years ago (1906) cultures of trypanosomes were valueless according to him. Now they are quoted as evidence. Are opinions based on such "weathercock" mental attitudes to be foisted upon the scientific public only to be abandoned in a short period when the writer has once more changed his views?

Dr Woodcock again states that his opinion is also "the opinion of the great majority of Protozoological workers." Up to the present, I am not aware that a poll has been taken of the workers on the Trypanosomatidae regarding this subject, but I could name at once at least six workers on this group, of international reputation, who certainly do not agree with that form of fallacy due to illicit generalisation that is so characteristic of Dr Woodcock's views, and who prefer facts to wild hypotheses. If appeal were made to them on the question whether flagellates occurring in blood-sucking insects were merely developing forms of trypanosomes, they would at once return the verdict "not proven." From recent work it seems that evidence is steadily accumulating that natural flagellates of blood-sucking insects are more common than was supposed. The work of Bruce and his colleagues on the *Crithidia* of Tabanids and of Wenyon on Herpetomonads in *Stomoxys* may at once be cited. The latter states that *Stomoxys*, "occasionally harbours a *Herpetomonas*, evidently a parasite peculiar to itself" (p. 107).

Reference has been made by Dr Woodcock in his reply to the article on Haemoflagellates which he contributed to Lankester's *Zoology*. One would like to enquire what Dr Woodcock's views were at the time that article was written, seeing that in more than one place therein, the names *Crithidia* and *Herpetomonas* are confused and placed as synonyms for one another. Very little has been said about *Herpetomonas* in Dr Woodcock's reply, yet the treatment allotted it was every whit as reprehensible as in the case of *Crithidia*.

Further, no mention whatever is made of the *International Catalogue of Scientific Literature*, but there is a somewhat defiant statement regarding Dr Woodcock's future actions in the *Zoological Record*. His future procedure in that direction is no concern of mine in this article. If he likes to continue to spoil the *Zoological Record* by personal interpretations based on illicit generalisations, and the managers of the Record allow it, that will, in the end, harm few but himself and the Record. It is a matter for congratulation that there is still an *International Catalogue of Zoological Scientific Literature* and a

Bibliographia zoologica, in the latter of which, at any rate, meddlesome Recorders are not allowed to air their views.

Dr Woodcock again by conjoining his opinions of me with those he holds of a most distinguished worker—an investigator to whom all workers on insect flagellates are for ever indebted—merely exposes again his ignorance of logic. Were I to reply to Dr Woodcock's discourteous remark (p. 715 of his paper on Avian Haemoprotozoa), I fear I should be under the painful necessity of emphasising strongly his ignorance of certain matters on which he claims by his procedure to be an authority. However, I should like to voice the opinion of myself and other workers by quoting Swingle (p. 127), "Patton's works...on *Crithidia* and *Herpetomonas* have given such complete knowledge of their life histories that it is now possible to undertake upon sure footing the investigation of the changes which trypanosomes undergo in their intermediate hosts. Patton's criticisms deserve the highest praise for the part they have served in showing the imperative care that must be exercised against drawing unwarranted conclusions."

Before concluding I should like to remark on a general proposition put forward by Woodcock in his paper on Avian Haemoprotozoa (p. 711), "Parasites exhibiting a trypaniform condition in a blood-sucking insect must be considered as belonging to the life cycle of a vertebrate trypanosome until the contrary is definitely established." I consider that this proposition is quite unnecessary. Dr Woodcock may try to justify it by referring to his definition of "trypaniform[1]" (a thoroughly bad term, be it noted, for it is a Graeco-Latin hybrid). The definition of trypaniform given is a certain condition characteristic of a trypanosome in which the kinetonucleus[1] lies much nearer to the aflagellar end than does the trophonucleus. Accepting his definition of "trypaniform," his general proposition is mere tautology. Most zoologists and certainly all protozoologists have seen trypanosomes and are acquainted with their form, so that there is no need of this attempt to foist a so-called general proposition of useless verbiage on a long-suffering scientific public. I am very sorry to see this verbiage copied into the *Bulletin of the Sleeping Sickness Bureau* (May, 1911) and placed in italics therein. A trained protozoologist would not have wasted valuable space in reprinting such meaningless verbosity.

[1] "Kinetonucleus" and "trophonucleus" are two more examples of Dr Woodcock's powers of coining terms, as well as further examples of his etymological attainments and fondness for ugly hybrids.

In conclusion, I am very sorry to be forced to state this, but the fact of the matter is that Dr Woodcock has lost the perspective of his subject. He has worshipped so long at the shrine of Schaudinn and worked on cultures of trypanosomes that his view is narrow and biassed. He generalises on one or two instances, as witness the general hypotheses he raises on the investigation of the life history of *T. lewisi*, and having proceeded to generalise, he cannot resist meddling with the work of others. I would suggest that the study of a standard work on Logic, particularly Inductive Logic, and especially the chapters dealing with Fallacies and Generalisations would be of distinct value to the gentleman who so airily talks of "logical views," "onus probandi," etc. However, I cannot further waste my time in dealing with wild hypotheses founded on a basis of illicit generalisations.

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This discussion is herewith closed.—*Ed.*

AN EXPERIMENT IN FUMIGATION OF TICKS.

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So far as I have been able to find, very little work, if any, has been done to ascertain the resistance of ticks to the gases usually employed in fumigation. This is probably because fumigation for the destruction of ticks is not, under ordinary conditions, a practical method. There recently arose, however, a question at Lourenço Marques (Portuguese East Africa), as to whether ships bringing cattle from Madagascar could be cleaned of the ticks which might have dropped from the cattle and be thus rendered clean for use in the transport of clean cattle for breeding purposes. The port authorities possessed a "Clayton Fumigating Apparatus" for use in disinfecting plague-infected ships and it was suggested that this system be tried for the purpose. Several tests were accordingly carried out with the co-operation of the Chief of the Veterinary Service, the Port Doctor, Municipal Officer of Health, and several local medical men. The "Clayton" being a standard system of fumigation, I will not enter into a discussion of it.

The management of the Clayton machine was left to the Port Doctor while the arrangement of the tests as regarded the ticks was in the charge of the writer. The ticks were arranged in several ways which we considered to approximate to the conditions under which ticks would be when they had fallen from cattle and crawled under débris and into cracks and crevices in the hold of a ship. Some of the tests were made as severe as possible and were severer than would ordinarily be met.

The first test was made on January 9th, but owing to a misunderstanding the hold of the ship where the test was made was not exposed to the gas for a sufficient length of time. Plague-infected ships are, I believe, exposed to the gas for six hours, counting from the time

when the sulphur has ceased to burn and employing a 10 % gas. On this occasion the time of exposure was measured from the moment when the sulphur began to burn, and, as sulphur requires about two to three hours to burn, the actual exposure to the 10% gas was only about three hours. This test, however, gave us some useful results upon which to base our future investigations. It was impossible to obtain a large number of species of ticks, owing partly to the season being unfavourable for excessive development of ticks. Large numbers of the Blue Ticks (*Boophilus decoloratus,* part of which were probably *B. australis*), and some Black-pitted (*Rhipicephalus simus*), Bont (*Amblyomma hebraeum*), and Red-legged Ticks (*Rhipicephalus evertsi*) in various stages were secured.

The ticks from this test were kept for fifteen days during which several examinations were made. Many ticks were found alive. In each case it was only the engorged females or nymphs which survived the effects of the gas. Unengorged females and nymphs or males perished in every case even where they were hidden in places which would seem almost inaccessible to a gas placed in the hold with so little pressure. One apparent exception to this occurred. A male *Amblyomma hebraeum* survived from among a lot of ticks placed in an open glass jar and hidden under a pile of iron bars. The next day, however, it died. The gas at 10 % and with this short exposure did not seem to be able to penetrate very deeply through obstacles nor did it seem to be of equal strength in all parts of the hold. It was decided, therefore, to try a new test, employing the gas at 15 % and giving an exposure of twelve hours. The ticks were prepared in the same manner as on the former occasion except that more species were employed and more stages of the various species.

The application of the gas was started on February 14th at 4 p.m. and continued until 2 % of gas was in the hold of the ship which was used for the experiment. A storm then arose making it necessary for the Clayton to disconnect. The hold of the ship was left partly closed until the next day at 4 p.m. when the Clayton was once more connected, the hold closed and all openings calked, and the gas forced in until it reached 15 %. It was left for twelve hours, at the expiration of which time it was aired for several hours and the ticks were removed. They were taken from their wrappings and placed in glass jars. These were left until March 7th during which interval several examinations were made. I will detail the manner of arranging the ticks with the species and stages employed and the results obtained.

The following species of ticks were secured :

Blue Tick, *Boophilus decoloratus.*
Bont Tick, *Amblyomma hebraeum.*
Red-legged Tick, *Rhipicephalus evertsi.*
Brown Tick, *Rhipicephalus appendiculatus.*
Black-pitted Tick, *Rhipicephalus simus.*
Tampans, *Ornithodorus moubata.*

The Blue Tick was secured in all stages—eggs, unengorged larvae, a few unengorged nymphs, unengorged and engorged females, and males. The Bont Ticks were secured as eggs, unengorged nymphs, engorged and unengorged females, and males. The Brown Ticks were all unengorged females and males. The remainder of the species were males and engorged and unengorged females. So far as possible the specimens were divided so as to have a few of each species and of each stage in each portion of the test. Check lots of the eggs used were kept apart to make sure of their vitality.

The tests were arranged so as to approximate as far as possible to the conditions which would exist in the hold of a cattle ship.

One lot of ticks was placed in an open glass fruit jar with mosquito netting tied over the top so as to prevent the escape of the ticks but allow free entrance to the gas. This was then placed in the centre of the floor. With it were also placed the following preparations : ticks in a glass fruit jar with butter cloth tied over the top ; a fruit jar filled with a sheet of cotton wool in the middle of which the ticks had been placed and then rolled up and tightly packed into the jar ; a paper shoe box containing ticks wrapped in three sheets of brown wrapping paper, one wrapped outside the other ; a glass fruit jar with ticks in the bottom and packed to the top with crumpled brown wrapping paper ; a fruit jar containing ticks and with mosquito netting tied over the top, but turned upside down on the floor ; a glass fruit jar, with ticks, over the top of which was tied a piece of bleached white calico ; and a fruit jar containing ticks but filled with crumpled brown wrapping paper and with a piece of butter cloth tied over the top. There were also prepared two pairs of boards about a foot and a half square. Between the first pair was packed hay to about a half-inch thickness, between the second pair was packed about the same thickness of cotton wool. In the centre of the packing of each were placed the ticks, tied in a piece of mosquito netting to prevent their escape. Another lot of ticks was hidden in the centre of a bale of hay purchased on the market. Other lots were tied in butter cloth and sacking and hidden under débris. One lot was placed in an old sack partly filled with hay. A box about one foot square by a foot and a half long was filled with dry sawdust and a lot of ticks tied in a piece of mosquito netting hidden in the centre after which the cover was tied on. Other lots of ticks were placed in small glass honey jars, over the tops of which mosquito netting was tied, and hidden under coils of rope, sails and débris found

in the hold of the ship used. In addition to this several honey jars containing engorged female Blue Ticks, and with mosquito netting tied over the tops, were placed in various parts of the hold, top and bottom, fore and aft, to ascertain whether the gas was of equal strength in all parts.

In this experiment all the ticks perished except one engorged female *Amblyomma hebraeum*, from the preparation where the ticks were placed between boards with hay packed between. This tick appeared dead at first, but subsequently produced a quantity of eggs nearly normal in number, although she did not retain vitality enough to move about. The eggs were kept under observation and several of those first laid hatched, while at the last examination the developing embryos could be seen in many others. The percentage of eggs retaining their vitality was, however, comparatively small. The eggs subjected to the gas never hatched but soon shrivelled up, whereas the check lots hatched in due time.

Owing to the fact that the ticks in the above experiment had been unavoidably subjected to the Clayton gas for more than twelve hours it was determined to try another smaller experiment to see if twelve hours' exposure to 15 % of the gas was quite sufficient to destroy the ticks. Accordingly the test was made on March 6th. The species and stages of ticks employed were the same as in the previous test. Only the preparations with ticks in the bale of hay, box of sawdust, wrapped in three sheets of brown wrapping paper and placed inside a shoe box, and the jar containing ticks wrapped in cotton wool, were employed. On March 23rd after several examinations it was found that every tick had been killed.

We must conclude from these trials that the Clayton gas (SO_2) is effective in killing ticks in the holds of cattle ships, when closed tightly, and is able to penetrate fairly dense substances. While, however, it is effective in destroying or preventing movement of live ticks, it is not absolutely effective in destroying entirely the vitality of engorged female ticks, so that an occasional one may survive to lay eggs, a portion of which may hatch. The system is therefore effective in cleaning ships from diseases which are transmitted from one stage of the ticks to the other, but where the disease can be transmitted through the eggs it is not entirely effective.

THE GEOGRAPHICAL DISTRIBUTION OF *ORNITHODORUS MOUBATA* (MURRAY, 1877).

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With a Map.

A PRECISE knowledge of the geographical distribution of *Ornithodorus moubata* is very desirable, as it bears directly upon the distribution of the Relapsing Fever of Tropical Africa. It therefore appears desirable to publish the accompanying map and a list of places showing the distribution of the tick. Both the map and the list are, however, of necessity far from perfect.

Although the number of States from which *O. moubata* has been recorded shows that the tick possesses a very wide distribution, the number of definitely recorded localities is small. It is therefore to be hoped that the empty appearance of the map may help to stimulate others to record *exact localities* relating to the distribution of *O. moubata*, and by so doing to augment our, at present, very meagre knowledge of the distribution of the tick. *O. moubata*, in general terms, may be said to extend southward from Lat. 10° N. and eastward from Long. 10° E., it is, however, from Uganda, the Congo Free State, British Central Africa and Portuguese West Africa that it is chiefly recorded. It occurs from Central Abyssinia to the Transvaal and Orange River Colony[1] in the East, across the Continent to the Congo, and from there southwards to German South-West Africa and the North of Cape Colony. It is

[1] Now known as Orange Free State.

Cambridge University Press

Map showing the geographical distribution of *Ornithodorus moubata*.

Table showing the places from which O. moubata *has been recorded, together with the name and date of the collector or reporter and the authority for the identification.*

State	Locality	Collector or recorder	Date	Identifier
Abyssinia ...	Harar	A. Brumpt	v. 1901	Nuttall.
	Galla	,, ...	1901	,,
	Biacobaba	,, ...	,,	,,
British E. Africa ...	Kilima-ndjaro	A. Brumpt	...	Nuttall.
	Pokomani (Wituland) ...	W. Dönitz	1906	Dönitz.
	Nairobi	P. H. Ross	x. 1908	Nuttall.
	,, ...	H. G. Simpson ...	1907	Merriman.
Uganda	Usoga	Christy	1903	Nuttall.
	Wadelai	,, ...	,,	,,
	Katwe	,, ...	,,	,,
	Toru	,, ...	,,	,,
	Mpumu (Chagwe)	P. H. Ross ...	vi. 1909 x. 1909	,,
	Mbale	H. G. Simpson ...	1907	Merriman.
	Entebbe	,, ...	,,	,,
	Bukoba	,, ...	,,	,,
	Mengo	,, ...	,,	,,
	Lake Mamba	,, ...	,,	,,
	Ngomanene	,, ...	,,	,,
	Mwangas Bugungu	E. D. W. Greig ...	1904	Neumann (Brit. Mus.).
	Mengo	A. B. Cook	viii. 1908	Nuttall.
	Buda	Christy	1903	,,
	Bussu	H. Bayon	x. 1909	,,
	Jinja	E. B. Haddon ...	x. 1906	,,
	Masaka	... ...	...	Robinson.
German E. Africa ..	Dar-es-Salaam	R. Koch	1904	Neumann.
	Kilossa	,, ...	,,	,,
	Mpapua	,, ...	,,	,,
	Tringa	,, ...	,,	,,
	Tabora	Dutton & Todd (*Report*)	1905	Newstead.
	Rubeho Mts.	R. Koch	1905	Neumann.
	Morago	... ...	...	Dönitz (Berlin Mus.).
	Teita	... ...	...	,, ,,
	Quange	... ...	...	,, ,,

Portuguese E. Africa	Tete	Ex Daniels coll. ...	1905	Nuttall.
	,, ...	D. Livingstone (*Report*)	1857	—
	Mozambique	F. C. Wellman ...	1908	Nuttall.
	Inhambane	C. W. Howard ...	VI. 1909	,,
	Komati Port	,,	...	Howard.
	Mopea	,,	...	,,
North-East Rhodesia	Near Chertekas, Luangwa River	S. A. Neave[1] ...	10. IX. 1910	Nuttall.
	Fort Jameson	,, ...	IX. 1910	,,
North-West Rhodesia	Sescheke	Sir John Kirk (*Tropical Diseases*, Manson) ...	1903	
South Rhodesia ...	Mt. Chirinda (Melsetter Dist.) 3800 ft.	Swynnerton ...	VIII. 1909	Nuttall.
British Central Africa	Lilongwe	C. W. Howard ...	VI. 1909	Nuttall.
Angoniland	,, ...	Ex Daniels coll. ...	III. 1907	,,
Nyasaland	Blantyre	... ...	1908	,,
	Ngara (Marimba Dist.) ...	H. Hearsey ...	X. 1909	,,
	Dowa Boma	E. H. A. Pask ...	III. 1909	,,
	,, ,, ...	H. Hearsey ...	III. 1909	,,
	,, ,, ...	Ex Daniels coll. ...	1905	,,
	Fort Hill	J. B. Davey ...	IV. 1909	,,
		S. A. Neave[1] ...	VI. 1909	,,
	Boma (Deep Bay)	,, [1] ...	X. 1909	,,
	Karonga	,, ...	VI. 1909	,,
	Hora	H. S. Stannus ...	X. 1906 / VI. 1909	,,
	Zomba	J. E. S. Old ...	VIII. 1906	Neumann (Brit. Mus.).
	Bua River (Marimba Dist.) ...	H. S. Stannus[1] ...	1910	Nuttall.
Transvaal ...	Lydenburg	A. Edington ...	...	Neumann (Brit. Mus.).
Orange River Colony	Koffiefontein	Government Biologist ...	1906	Neumann.
Zanzibar	Zanzibar	E. Brumpt	...	Nuttall.
Cameroons ...	S. Lake Tchad	Closel	...	Neumann.
French Congo ...	Loango	Foà	1901	Neumann.
Congo Free State ...	Myangwe	D. Livingstone (*Report*)	1874	
	,, ...	Dutton & Todd (*Report*)	1905	Newstead.
	Kasongo	,, ,,	,,	,,
	Lokandu	,, ,,	,,	,,
	Mulamba	,, ,,	,,	,,

[1] Collecting on behalf of the Entomological Committee (Tropical Africa) acting for the Colonial Office.

Table showing the places from which O. moubata *has been recorded, etc. (continued).*

State	Locality	Collector or recorder	Date	Identifier
Congo Free State (*cont.*)	Ukungwa	Dutton & Todd (*Report*)	1905	Newstead.
	Mwana Maketa	,, ,,	,,	,,
	Piani-lusani	,, ,,	,,	,,
	Kabambare	,, ,,	,,	,,
	Mifuso...	,, ,,	,,	,,
	Albertville	,, ,,	,,	,,
	Baudovinville	,, ,,	,,	,,
	Mpala	,, ,,	,,	,,
	Lake Kabele	,, ,,	,,	,,
	Katola	,, ,,	,,	,,
	Popokabaka	,, ,,	,,	,,
	Francis Joseph Falls ...	,, ,,	,,	,,
	Kimpudi	,, ,,	,,	,,
	Uvira	,, ,,	,,	,,
	Rutschuru	,, ,,	,,	,,
	Beni	,, ,,	,,	,,
	Ponthierville	,, ,,	,,	,,
	Kirundu	,, ,,	,,	,,
	Mashi Kibangu	,, ,,	,,	,,
	Fuma Lumbo	,, ,,	,,	,,
	Muene Lubanda	,, ,,	,,	,,
Portuguese W. Africa	Ambaca...	D. Livingstone (*Report*)	1857	
	,, ...	F. C. Wellman ...	1908	Nuttall.
	Bihé	,, ...	,,	,,
	Bailundo	,, ...	,,	,,
	Kakonda	,, ...	,,	,,
	Benguella	,, ...	v. 1906 v. 1908	,,
	Landana	,, ...	1908	,,
	Malange	,, ...	,,	,,
	Audulo	,, ...	,,	,,
	Chiyaka	,, ...	,,	,,
	Loraleland	,, ...	,,	,,
	Moxico	,, ...	,,	,,
	Kuanza...	... ...	1901	Neumann (Brit. Mus.).

only recently that *O. moubata* has been distinguished, with certainty, from *O. savignyi* and therefore many of the older records are not to be relied on. The accompanying map only records those places (⊙) from which ticks have been received, or recorded, by trustworthy observers, the regions, denoted by the sign ×, are those in which it is practically certain that *O. moubata* does occur.

The appended list includes all the places from which *O. moubata* has been recorded. (It contains names of places which are not marked on the map because of their precise position being doubtful.) Wherever possible the list includes the names of the collectors, the date of the record and the authority by whom the specimens were identified. It comprises all the places mentioned by Nuttall and Warburton[1] (1908), and includes additional data relating to specimens received during the last two years at the Quick Laboratory, and information obtained through our correspondents—Prof. Dahl (Berlin Museum), Prof. Bouvier (Paris Museum), Prof. Newstead (Liverpool School of Tropical Medicine), Mr E. B. Haddon (Uganda), Mr L. E. Robinson (Watford), and Mr H. G. Simpson (Nairobi), to all of whom we are indebted for the information they have kindly supplied. I have moreover re-examined the specimens (determined as *O. savignyi* var. *caeca* by Prof. L. G. Neumann) which are in the British Museum.

[1] *Ticks*, Part I, pp. 52–54. The references to the literature will be found in this publication.

NOTE ON ENTOMOLOGICAL BOXES.

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In *Parasitology*, Vol. III. p. 486, there is a description by F. M. Howlett of the paraffin boxes in use here for preserving pinned insects. As the description of these boxes is incomplete and not quite correct, it seems desirable to add a description which will enable others to make these boxes. We use teak boxes, of a standard size, well varnished within; when the varnish is dry, a piece of cork carpet cut to fit the box and previously enamelled white above, is laid ready to hand, a mixture of paraffin wax (melting point 136° F.) 80 % and flake naphthaline 20 % is kept melted and a small quantity is run into the box; the cork carpet is at once put in, and rolled down on the melted mixture which sets and holds it; more of the liquid is then run in, sufficient to cover the cork completely; this sets with an even smooth surface (the box being on a perfectly horizontal surface), and the box is ready when cold. This method has not only been tried but we have nearly 2000 boxes in use and are replacing all our papered boxes. To all who may wish to preserve insects in one place I recommend these boxes, but they are too heavy if one is moving and for that a lighter paraffined box is required.

Let me point out that the improvement in our method consists in replacing papered cork (which gets discoloured and requires preservatives) by paraffined cork which requires no poisoning, which keeps white and offers no harbourage to any insect. We have had these boxes in use for two rainy seasons and they have given complete satisfaction, without the addition of creasote or any preservative, and the surface remains white.

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